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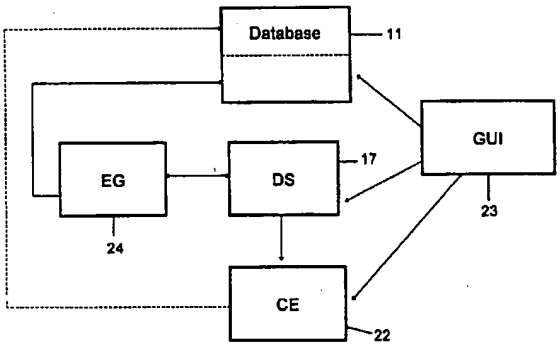

(57) Abstract: Genetic, biochemical, biophysical and anatomical information is integrated at the subcellular, cellular, tissue and organ level. At least one database containing biological information is used to generate at least one data structure having at least one attribute associated therewith. An interface interactively views, edits or links together attributes of the data structures to create at least one hierarchical description of subcellular, cellular, tissue and organ function. The hierarchical description may optionally be an elementary, binary or pathway data structure, or, alternatively, an anatomical data structure capable of being modified to form a structural model. A computational engine mathematically generates at least one data structure from the hierarchical description. Genetic information is accessed, tabulated and combined with functional information on the biochemical and physiological role of gene products. Computational models of genetic, biochemical and biophysical processes within cells and higher order systems are automatically formulated, solved and analyzed based on combination of genetic and functional information adduced. A dynamic tool is thereby provided for achieving discernible objectives, such as increased understanding of biological processes, identification of new drug targets for therapeutic intervention and predictions involving the outcome of drug screening. These objectives are accomplished by the realization of highly complex nonlinear dynamic interactions that occur between each gene or gene product.

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SYSTEM AND METHOD FOR MODELING GENETIC, BIOCHEMICAL, BIOPHYSICAL AND ANATOMICAL INFORMATION: IN SILICO CELL

This is a continuation-in-part of Application Serial No. 09/295,503, filed April 21, 1999, which claims the benefit of U.S. Provisional Application No. 60/083,295, filed April 28,1998.

BACKGROUND OF THE INVENTION

1. Field of the Invention:

The present invention relates to a computer-implemented system of constructing databases and modeling biological processes; and more particularly to mathematical, informational, and computational processes and procedures for automatically generating computer-based models that integrate biological information from the subcellular to the cellular, tissue and organ level.

2. Description of the Prior Art:

Cell biologists face a major challenge distilling the vast quantity of new data that is being generated at heretofore unprecedented rates. At present, hundreds of biological databases are listed in DBCAT, the INFOBIOGEN biological database catalog accessible from the World Wide Web (http://www.infobiogen.fr/services/dbcat/) and available publicly through the National Center for BioTechnology Information (http://www.ncbi nlm.nih.gov). This information explosion has been driven by the continuous development of information technology such as the Internet as well as the development of powerful new technologies for automatically collecting and storing data such as in gene sequencing and gene expression profiling. These databases contain genomic, biochemical, chemical and molecular biology data as well as structural data comprising geometric and anatomical information from the subcellular to the whole organism level. Some of these data are organized by data type including, for example, the International Nucleic Acid Sequence Data Library (a.k.a. GenBank) and NAD for nucleic acid sequences;

SWISS-PROT for protein sequences; PDB for protein structures and the like. Other databases are organism specific and include GDB and OMIM for human; MGD for mouse, PigBASE for pig; ATDB for *Arabidopsis;* ECDC for *E. Coli,* and many others. Still other databases contain information on particular areas of interest, such as specific databases for individual genes, databases about specific protein families, and databases of transcription factors. Biochemical databases contain information regarding coupled biochemical reactions and feedback signals which take place within the cell. Additionally, proprietary databases such as the availability of entire genomic sequences due to improved high throughput gene sequencing, available from the large data production houses, have been created and are expanding with technology.

Substantial work is underway to integrate data from these diverse databases. *See e.g.,* Macauley, et al., A Model System for Studying the Integration of Molecular Biology Databases, 14 *Bioinformatics* 575-582 (1998).

Efforts to organize and analyze the vast amount of genomic data have stimulated the development of a new field of computational science known as bioinformatics; the science of using computers and software to store, extract, organize, analyze, interpret and utilize gene sequence data to identify new genes and gene function- in order to understand the genetic basis of disease and to further gene-based drug discovery and development. This approach typically uses a one-dimensional computational analysis to study explicit information about the genome such as percentage of gene sequence similarity across species, homology of sequence motifs across species, expression levels in various tissue types, secondary structure correlations, etc. Although the acquisition of genomic information is clearly essential, there is growing recognition that conventional methods are insufficient for correlating that information with the functional role of genes and gene products. Rather, in all cells, genetic expression produces self-organizing networks controlling cell functions, including developmental pathways, progression through cell cycle, metabolism, intracellular signaling, cell excitability and motility, and

feedback loops regulating gene expression. At present, bioinformatics is unable to simulate these complex, highly nonlinear dynamic interactions that occur between each gene or gene product, and other components of the network they are a part of. Thus, bioinformatics researchers do not, at present, have the necessary tools to obtain a complete representation of subcellular and cellular processes, as well as the effect of these processes on tissues and organs.

One approach to dealing with these complex, highly nonlinear interactions has focused on computational modeling. There is an extensive 40 year history of such modeling that includes simple models with a few state equations that describe processes within cells to highly complex models of organ systems that must be implemented on high performance multiprocessor computers (Rall W., Burke R.E., Holmes W.R., Jack J.J., Redman S.J., Segev I. (1992) *Physiol. Rev.* 72(4 Suppl) 5159-86; Rall W. (1967) *J. NeuroPhysiol* 30(5): 1169-93, Segev I. and Rall W. (1998) *Trends Neurosci* 21(11): 453-60; Koch C., Poggio T., and Torre V. (1982) *Philos. Trans. Roy. Soc. Lond. B.* 298(1090):227-63, Chay T.R. and Rinzel J. (1985) *Biophys. J.* 47(3): 357-66; Smolen P., Rinzel J., Sherman A. (1993) *Biophys J.* 64(6): 1668-80, Shepherd G.M.et al (1998) *Trends Neurosci* 21(11): 460-8). This approach provides a means to link experimental data regarding specific biological processes to cell function. The culmination of this 40 year history can be seen in several efforts such as the nationally funded efforts, The Human Brain Project and the Virtual Cell Project. The Human Brain Project is a multi-agency funded multi-site effort to organize and utilize diverse data about the brain and behavior. The Virtual Cell project has developed a framework for organizing, modeling, simulating, and visualizing cell structure and physiology. However, these projects lack an overall ability to link to existing genetic, protein and structural data bases. In addition, these projects have not defined procedures for modeling biological systems using information stored in local or distributed databases. As such, detailed and accurate representations of the many different simultaneous subcellular and cellular processes and the effect

of these processes on cellular systems which occur at any given time are not presently possible.

What is needed therefore are new computer based tools to formulate computational models of subcellular and cellular processes, as well as the effect of these processes on intercellular systems. Such tools will provide a means for linking information at the level of the gene to functional properties of intercellular systems in health and disease, will further the understanding of disease processes, and aid in drug target identification and screening.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a system and method for integrating genetic, biochemical, biophysical and anatomical information at the subcellular, cellular, tissue and organ level. Generally stated, the system comprises: (a) at least one database containing biological information which is used to generate at least one data structure having at least one attribute associated therewith; (b) a user interface for interactively viewing and editing attributes the data structure to create at least one hierarchical description of subcellular, cellular, tissue or organ function; (c) an equation generation engine operative to generate at least one mathematical equation from at least one hierarchical description; and (d) a computational engine operative on at least one mathematical equation to model dynamic biological behavior.

Advantageously, the system of the present invention can access and tabulate genetic information contained within proprietary and nonproprietary databases, combine this with functional information on the biochemical and biophysical role of gene products and based on this information; formulate, solve and analyze computational models of genetic, biochemical and biophysical processes within cells and higher order biological systems. The system of the invention therefore provides a dynamic tool for quantitative understanding of biological processes, identifying new drug targets for therapeutic intervention and predicting the outcome of drug

screening. This is accomplished by the accurate modeling and simulation of highly complex nonlinear dynamic interactions that occur between each gene or gene product.

In another aspect of the invention there is provided a method modeling biological information that accounts for multiple time frames inherent in biological processes comprising: (a) at least one database containing biological information which is used to generate a plurality of data structures, each having at least one attribute associated therewith; (b) a user interface for viewing, editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system; (c) a correlation engine operative on at least one hierarchical description of a biological system to generate a simplified system of equations; and (d) a computational engine operative to solve the simplified system of equations to create a model of a dynamic biological process. The models created in accordance with this method integrate biological knowledge across all levels of analysis ranging from that of the gene to that of the cell, tissue and organ to provide a detailed and accurate representation of heterogeneous systems. This integration provides a multi-dimensional analysis which simply was not possible with the one-dimensional genomic computational analysis tools of the prior art.

In yet another aspect of the present invention there is provided a method for creating a model of biological information for use with a computer system, comprising: (a) accessing at least one database containing biological information; (b) generating a plurality of data structures, each having at least one attribute associated therewith; (c) interactively viewing editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system; and (d) utilizing a at least one computational engine to mathematically generate at least one model of a biological system reflective of the multiple time frames inherent in biological processes.

In still another aspect of the invention there is provided a method for linking models of subcellular and cellular processes to systems processes comprising: (a) generating at least one hierarchical description of

subcellular function from at least one database containing biological information, the hierarchical description generated from a data structure having at least one attribute associated therewith; (b) generating at least one hierarchical description of cellular function by linking a plurality of attributes of subcellular function from the hierarchical description of subcellular function; (c) generating at one least hierarchical description of system function by linking a plurality of attributes of cellular function from the hierarchical description of cellular function; and (d) utilizing at least one computational engine to mathematically generate at least one model of a biological system reflective of a biological system. Advantageously, this allows for the creation of highly complex models of biological systems.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be more fully understood and further advantages will become apparent when reference is made to the following detailed description and the accompanying drawings in which:

FIG. 1 is a schematic diagram illustrating the overall flow of operations through the system of the present invention;

FIG. 2 is a Pathway Data Structure depicting the topology of the pyruvate dehydrogenase reaction in which pyruvate is converted to acetyl-CoA;

FIG. 3 is a block diagram illustrating the flow of information to produce hierarchical descriptions of subcellular and cellular function in which EDS defines an elementary structure, BDS defines a binary data structure, and PDS defines a pathway data structure;

FIG. 4 depicts a Binary Data Structure;

FIG. 5 illustrates a Binary Data Structure modeling a biophysical process;

FIG. 6 illustrates a Binary Data Structure representing a gene regulatory network;

FIG. 7 is a schematic diagram illustrating the flow of information used to generate structural, finite-element cell models;

FIG. 8 illustrates a biochemical reaction network;

FIG. 9a illustrates a naive (quiescent) signal transduction pathway for P13 kinase in Tcells;

FIG. 9b illustrates activation of a signal transduction pathway for P13 kinase in Tcells;

FIG. 9c illustrates inhibition of a signal transduction pathway for P13 kinase in Tcells;

FIG. 10 sets forth a model of Tcell differentiation in rheumatoid arthritis;

FIG. 11 sets forth a model of inhibition of Tcell differentiation in rheumatoid arthritis as a result of TNF-α therapy;

FIG. 12a sets forth a model of Tcell differentiation from T0 to Th1;

FIG. 12b sets forth a model of Tcell differentiation from T0 to Th2;

FIG. 13 provides an example of a descriptive report generated by the system of the invention in response to a specific modeling query;

FIG. 14 provides an illustrative graphical model output for the dynamic change in concentrations or levels in a T-cell that is characteristic of the behavior of that cell, and is characteristic of the signaling within the T-cell.

FIG. 15 illustrates the various reaction pathways involved during the activation of Tcells;

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a multidimensional computational tool capable of integrating biological knowledge across all levels of analysis ranging from that of the gene to that of the cell, tissue and organ. This is accomplished by a system and method which incorporates at least one database that stores biological information, an interface which displays, links, organizes and modifies that information, and computational

engines which operate on the information contained in the database to automatically formulate, solve and analyze computational models of biochemical reaction networks, biophysical mechanisms, and in general dynamic processes at the subcellular, cellular, tissue, and organ level.

More specifically, the present invention is an interactive computer-implemented system for mathematically modeling biological information from the subcellular to the cellular, tissue, and organ level comprising: (a) at least one database containing biological information which is used to generate a plurality of data structures having at least one attribute associated therewith; (b) a user interface for interactively viewing and linking together attributes the plurality of data structures to create at least one hierarchical description of subcellular, cellular, tissue or organ function; (c) an equation generation engine operative to generate at least one mathematical equation from at least one hierarchical description; and (d) a computational engine operative on at least one mathematical equation to model dynamic biological behavior.

The system of the present invention uses computer-implemented tools to link genetic and molecular information to the topological and kinetic properties of biochemical and biophysical processes within cells, tissues and organs, to provide functional information on the biochemical and physiological role of gene products, and the effect thereof on biological systems. This information is coupled to computational engines that can automatically formulate, interconnect, solve and analyze properties of computational models of genetic, biochemical and biophysical processes within biological systems. In this way, it is possible to address the functional role played by each molecular/genetic component from which a model is composed, to identify optimal points of therapeutic intervention within these models and to "numerically screen" lead compounds for functional effects on these models.

Referring now to the drawings, there is shown in Fig. 1 a schematic diagram illustrating the overall flow of operations of the system of the present invention. Generally stated, the system includes database 11, data

structure 17, graphical user interface 23 for interactive contact with the information generated by the system, equation generation engine 24 and a computational engine 22.

Databases

Database 11 encompasses both internal and external databases. External refers to databases designed to store and organize biological information, but which were not designed explicitly to be coupled with the subcellular, cellular, tissue and organ modeling, simulation, and analysis tools described herein. Internal refers to databases with a specific structure (to be described in subsequent sections) which are designed explicitly to support the formulation, simulation, and analysis of subcellular, cellular and systems models. Internal and external databases include those containing gene and protein sequences, biochemical and biophysical processes, descriptions of cellular, tissue and organ physical structure, experimentally validated models of biochemical and physiological processes, or models previously generated by the system. Advantageously, database 11 may contain one or any number of the foregoing databases.

Any means for accessing and searching external and internal databases may be used in the present invention. Typically these would include: commercial database front-ends with SQL queries, web-based solutions such as Perl scripts and Java-based tools for accessing remote databases, as well as cross-platform software tools available, for example, from Genomica Corp. (Boulder, CO), Pangea Systems, Inc. (Oakland. CA) and NetGenics Inc. (Cleveland, OH).

Internal databases include those that have been generated from the data extracted from the external databases as well as data added by users via the graphical user interface. Such data may include experimental data including, for example, new descriptions of biochemical and physiological processes, or it may be data generated as a result of computer modeling by the system. Data generated and stored by the internal databases are manipulated using commercially available object-relational or relational database

management systems such as Oracle Corp. (Redwood City, CA), Sybase, Inc. (Emeryville, CA), or Informix (Menlo Park, CA), or using markup languages such as SGML or XML, all of which are well known to the skilled artisan. Most importantly, the internal databases store information on the (a) topology; (b) kinetics; and (c) interconnectivity between various genetic and biochemical reaction networks (BRN'S) within cells. These are generically referred to as internal biochemical databases herein.

In the context of the present invention, topology refers to the pattern of interactions within a specific genetic or biochemical reaction network; kinetics refers to the reaction rate constants that, in conjunction with the laws of mass action, determine the dynamic behavior of such reaction network processes; and interconnectivity refers to the specific points of coupling between different genetic and biochemical reaction networks within the cell which results in cellular behavior. Thus, the internal biochemical databases store the interconnection topology, including the rate constants associated therewith, for each BRN. By way of example, the BRN for the pyruvate dehydrogenase reaction in which pyruvate is converted to acetyl-CoA is illustrated in FIG. 2. Information on this BRN which is stored in the internal biochemical databases includes each of the intermediates involved in the reaction, the enzymes involved in determining the rate at which the intermediates are formed (along with lists of co-factors influencing the reaction rate such as pH, temperature, and the like) and the reaction pathways connecting these intermediates. These databases also include qualitative data such as cell-cell, cell-molecule, molecule-molecule interactions, cell growth rates, binding constants, concentration effects of cells and molecules on cell-cell, cell-molecule, and molecule-molecule interactions and the like. Advantageously, more than one BRN may be linked together to provide a more complex representation of subcellular, cellular and system behavior.

The internal biochemical databases store genetic and biochemical reaction network data in a way that makes possible the hierarchical construction of mathematical and computational models of these networks from their underlying components. Equation generation engine 24

transforms each genetic and biochemical entity within the internal biochemical database into a group of symbolic equations and numerical subroutines associated therewith which are stored as attributes of these entities. As discussed in more detail later, use of these attributes allows the user to simulate and view functional behavior of this entity (based on the genetic/biochemical properties of interest) by way of graphical user interface 23, and computational engine 22. In this way, the system makes it possible to link genetic and molecular information to functional information regarding subcellular, cellular and system processes. Preferably, each of these attributes associated with the genetic and biochemical entities also includes time delays in process through implicit time constants that are functions of kinetic rates. This allows a model to incorporate multiple time frames to account for disease progression in cellular and system models. The biochemical reaction networks (BRNs) can be compartmentalized, i.e., a set of BRNs can be gathered into different compartments each of which can have different attributes such as surface area, internal volume, geometry among others. This can create representations of different cells that have specific reactions and molecules that interact by crossing the compartment boundary. When a reaction carries a molecule from inside a compartment (cell) to its outside, and that molecule is then taken into another compartment (cell), the cells are communicating with each other and is one aspect of a tissue model. A molecule can sit on one compartment boundary and attach to another molecule sitting on another compartment and this represents cell-cell contact and is another aspect of a tissue model. This process can be built up by including all the cell types and their quantitative numbers and thus build a complete tissue. In the same hierarchical way, the tissue compartments can then be used to create whole organs.

A number of databases are presently available or are currently being developed, *see e.g.*, Popel et al., *The Microcirculation Physiome Project*, 26 *Annals of Biomedical Engineering* 911-913 (1998). These databases can be created and organized by known software tools which help users build and organize databases such as, for example. those available from

Oracle Corp. (Redwood City, CA). Software tools for designing and viewing, interactive graphical representations via graphical user interface 23 of these databases are also well known and readily available.

The internal databases will also represent and store information regarding biophysical processes within cells, tissues and organs. These internal biophysical databases contain information on the physical properties of biological processes required to formulate mathematical and computational models of these processes; for example, ion channels and currents, membrane transport systems such as pumps and exchangers, membrane receptors and signal transduction pathways for a given cellular process. Once formulated, each physical property stores as attributes a group of symbolic equations and numerical subroutines associated therewith which allow the user to simulate and view cell function (based on the biophysical properties of interest) via graphical user interface 23, equation generation engine 24 and computational engine 22. As above, these attributes may also include time delay in processes, enabling the incorporation of multiple time frames.

Internal databases also comprise internal structural databases which contain information on the physical structure and spatial relationship between various organelles within a given cell, as well as the relationships between cells in tissues and organs. Typically, this information is in the form of three-dimensional image data obtained from different modalities (e.g. electron micrograph serial sections, confocal serial sections, two-photon laser scanning serial sections, magnetic resonance images, position emission tomography images and the like. Optionally, the three-dimensional image data may be further transformed into structural finite-element models describing cell, tissue and organ shape and spatial placement of organelles and/or cells therewithin via an optional computational modeling engine which will be discussed in greater detail below. Structural models generated from the three-dimensional data are also stored in the structural databases. The structural databases thus contain information on anatomical subcellular, cellular, tissue and organ structure and spatial relationships which, in conjunction with the molecular, biochemical and biophysical databases,

provides the data necessary to produce a complete model of subcellular, cellular, tissue and organ function. As with other databases, the structural databases may be publicly available or it may consist of a novel or proprietary database.

By way of example, the precise geometry of and the spatial relationship between cardiac T-tubules and their associated L-type calcium ("Ca") channels and ryanodine-sensitive Ca release channels in the sarcoplasmic reticulum membrane provides information on the properties of calcium-induced calcium release, and therefore mechanical force generation in cardiac muscle cells. Likewise, information about the physical location of Ca-channels and Ca-modulated potassium channels in auditory hair cells provides information about the electrical tuning of these cells or knowledge of the spatial location of subcellular processes in specific cell organelles, e.g. mitochondrial respiration, provides the information necessary for a complete and accurate model of the entire cell.

External databases used in the present invention may be accessible through known commercial channels or the Internet. Typically, these databases contain gene sequence, protein sequence and three dimensional structural data on each constituent of a biochemical reaction network within a given cell or larger biological system, but certainly any type of data useful to develop models of subcellular, cellular, tissue and organ function is within the scope of the present invention. External databases such as those on the Internet are becoming increasingly standardized so that access to a variety of diverse databases is possible in a single application. *See e.g.*, Markowitz et al., Characterizing Heterogeneous Molecular Biology Database Systems, 2 *J. Comput. Biol.* 547-556 (1995). Advantageously, the system of the present invention accesses and utilizes data from the external databases during model creation. Alternatively, the system may transfer the information from these databases into another database (not identified) in the system for later use.

The information in internal database 11 is organized into and stored as at least one data structure which is used to construct at least one

model of subcellular, cellular, or systems processes. Preferably, the data structure comprises either a group of hierarchical description of subcellular, cellular and system function 17. Alternatively, the data structure comprises anatomical data structures describing the physical organization and structure of biological cells, tissues and organs.

Data Structures

Data structure refers to a group of interdependent data generated from information obtained from literature, experiments, expert information and internal information. Typically, data structures are constructed by means of the graphical user interface and the information available in the database 11. They may also be retrieved from previously defined data structures residing in database 11, or generated from biological inputs (e.g., experimental data) into the system. Graphical user interfaces and databases can in turn be developed using software tools such as those available from Microsoft (Redmond, WA) or Oracle Corporation (Redwood City, CA).

Referring to FIG. 3, data structure 17 comprises elementary data structure 16, binary data structure 19 and pathway data structure 2 with the binary 19 and pathway 20 data structure formed from the lower level data structures. The lowest level data structure is the elementary data structure ("EDS") 17. Each EDS 17 may comprise either a protein i.e., an entity coded by a gene, or a variable. As used herein, a variable refers to anything other than a gene, which defines interdependencies in cell processes as for example, elements or ions important to cell function such as K^+, Na^+, Ca^+, H^+, organic or inorganic compounds such as ATP, ADP, P_i, or any abstracted quantity describing the state of a biochemical or biophysical process, and which relates to organ, tissue, cellular, subcellular, molecular, or genetic function. EDS's may also comprise state variables, a set of parameters which allow the calculation of the behavior of the system at a point in time.

In accordance with the present invention, each EDS is associated with an extensive set of attributes. For example, attributes associated with a protein might describe the organism in which the protein is found, the specific

cell in which the protein is found, the specific gene coding for the protein, the sequence of the gene coding for the gene and so forth. The attributes describing each EDS are defined and hierarchically arranged by means of the graphical user interface 23.

These hierarchical description attributes thus comprise a grouping of pointers to specific portions in database 11 in which specific information associated with each attribute is found. By way of example, the attributes associated with a given protein could be arranged as Organism: Cell:Gene:State:Sequence:Structure:Location:Model. In this instance, the attribute "Organism" is a pointer to the appropriate gene database in which a gene which codes for the protein exists. The attribute "Cell" points to the specific cell type within that database in which the gene is expressed. The attribute "Gene" is a pointer to the specific gene in the database. The attribute "State" identifies the state of the Organism:Cell:Gene triplet and may be anything that might effect expression of the protein such as an age-related parameter, the presence of a particular disease in the organism, a particular time in the progression of a disease, or the like. Therefore, the attribute "State" is a pointer identifying which particular subset of the Organism:Cell:Gene database to search. The attribute "Sequence" is a pointer to sequence data in the structure of the gene coding for the protein. The attribute "Structure" is a pointer to the three-dimensional structure of the protein coded by that gene, if known. The attribute "Model" is a pointer to a database in which functional models of the protein coded by that gene are stored. Although reference has been made to protein-related attributes, any information regarding biological entities is within the scope of the present invention.

Binary data structure ("BDS") 19 is formed as a composition of more than one EDS. As more specifically illustrated in FIG. 4, BDS 19 comprises separate EDS's with arcs denoting the transitions between these EDS'S. In this example, EDS 1 represents the elementary data structure corresponding to state 1 of the binary relationship, EDS 2 represents an elementary data structure corresponding to state 2, and EDS 3 and EDS 4 are

elementary data structures determining the forward and backward transition rates, respectively, of the reaction between state 1 and state 2. This binary representation is also known as a state transition diagram. Thus BDS's are the first level data structures at which information on the topology and kinetics of biological reaction networks are represented. BDS's are generated from knowledge of biophysical and biochemical pathways within intra and intercellular systems. They may be derived from interrogation of existing biological databases, or may be generated using graphical user interface 23 from proprietary experimental data.

The binary relationship illustrated in FIG. 4 has many analogues in biological systems. For example, the binary relationship may represent transitions between two intermediates within the complex biochemical network shown in FIG 2. In this instance, EDS 1 could represent pyruvate (a variable), EDS 2 could represent Acetyl-CoA (a variable), EDS 3 could represent the catalytic enzyme pyruvate dehydrogenase (a protein), and EDS 4 could represent the substrate NAD (a variable). Alternatively, the binary data structure could represent a simple two-state closed-open model of a cardiac ion channel, thus modeling a biophysical process as shown in FIG. 5. In this instance, EDS 1 corresponds the closed state of an ion channel (a variable), EDS 2 corresponds to the open state of the ion channel (a variable), and EDS 3 and 4 would be identical and equal to membrane potential V (variables). The functional dependence of the transition rate constants K12 and K21 on quantities such as temperature, pH, membrane potential, and in general variables and/or proteins as defined previously, on membrane potential may or may not be specified, but the fact that a dependence exists would be. As another example, a binary representation of a gene regulatory network is shown in FIG. 6. Here, EDS I represents an RNA polymerase (protein), EDS 2 represents a closed RNA polymerase complex (variable), and EDS 3 represents a promoter (protein).

BDS 19 is also associated with a number of attribute lists. For example, the BDS in FIG. 4 may be represented by the list Input:Output:Frate:Brate wherein the attribute "Input" is associated with EDS

1, the attribute "Output" is associated with EDS 2, the attribute "Frate" is associated with EDS 3 and describes the forward transition rate, and the attribute "Brate" is associated with EDS 4 and describes the backward transition rate. As with the EDS'S, a graphical user interface 23, or an interface into existing biological databases 11. would be used to generate the linked attribute lists.

BDS 19 retains the attributes of each EDS which it comprises. The linked attribute lists defining BSD 19 would incorporate multiple attributes reflective of the group of attributes associated with each EDS. Therefore, a BSD may have distinct attributes of the Organism:Cell: Gene:State:Sequence:Structure:Location:Model attribute list discussed previously, but would not contain the single "Gene", "Sequence" or "Structure" attribute each is associated with a single EDS.

Pathway data structure ("PDS") 20 represents the highest level of data structure and is generated as the composition of more than one BDS. An example of a PDS is the pyruvate dehydrogenase reaction depicted in FIG. 2. As illustrated in FIGS. 9a, 9b and 9c, another example of a PDS would be detailed information pertaining to protein expression in three phases of a cell's existence: naive (quiescent), activated, and inhibited (for the naive or activated state). As another example, a PDS may comprise information regarding T-cell differentiation as is shown in FIG. 10. Thus, PDS 20 represents a more complex state transition diagram which retains the attributes of the EDS's and BDS's present in the pathway.

PDS 20 is also associated with a number of attribute lists. Because PDS 20 retains the attributes of its constituents, the attribute list Organism:Cell:-Gene:State:-Sequence:Structure:Location:Model described above may be applied to PDS 20. The modeling tools used to organize the databases and generate the EDS'S, BDS's and their associated data may be used to generate the PDS'S.

In accordance with the present invention, any biochemical reaction and physiological process can be arranged into an EDS, BDS and PDS and its associated attribute list. Typically, the data associated with the

data structures is generated by a user either prior to or at the time of model construction, or may comprise an attribute list from database 11 which is edited by the user. Advantageously, models are configured so that a user can interact with graphical user interface 23 to retrieve, view and edit any of the data associated with or generated by the data structures and their associated attribute lists to thereby create revised data structures and attribute lists. The structure of the attribute lists also permit a user to analyze multiple data structures to determine common and unique properties. With this information, a user can link attributes from more than one data structure to analyze common information or create detailed models of subcellular and cellular processes as well as of complex biological systems (e.g., organs).

Data structure 17 may also comprise at least one anatomical data structure describing the physical organization and structure of biological cells, tissues and organs. These data structures may be in the form of sets of three-dimensional image data from structural database as previously discussed.

Like the other data structures, the three-dimensional image data and the structural finite element cell models have specific attributes. Typically, these attributes are in the form Organsim:Cell:Organelle: Modality:ImageFormat, wherein the attributes "Organism" and "Cell" are as discussed above. "Organelle" is a pointer to that part of the anatomical database defining structure, "Modality" defines the type of anatomical data (such as a model derived from the three-dimensional image data or the three-dimensional image data itself), and "ImageFormat" defines the structure of the anatomical data. Optionally, the attribute "Organ" would be included.

As more specifically illustrated in FIG. 7, three-dimensional image data from structural database is defined by attribute lists 44. This three-dimensional image data may be further transformed by geometry modeling engine 42 into structural finite-element model 43 describing cell, tissue and organ shape and spatial placement of organelles and/or cells therewithin. which may be used to create additional list 45. Well known and readily available geometry modeling engines useful in the construction of

these structural models include EnSight (available from CEI, Inc., Research Triangle Park, NC) and FIDAP (available from Fluent Inc., Lebanon, NH). Each of the three-dimensional image data or the finite element models may be stored in the system for later use or generated as necessary. During the creation of a subsequent model, a user would have access to any of the three-dimensional image data from structural database 15, structural finite-element cell model 43, or attribute list 44 or 45. As such, the anatomical data structure may be specifically tailored to subsequent model use.

Preferably, the EDS, BDS and PDS's may be updated via a database interface, such as the i-Base interface proprietary to Physiome Sciences. Most preferably, a user can use the database interface to pose specific queries regarding biological processes to the system, analyze experimental data and hypothesize against known EDS's, BDS's and PDS's.

Computational/Equation Generation Engines

Generally stated, computational engines transform the data structures into mathematical models of biochemical, physiological and structural subcellular, cellular, tissue and organ processes. Advantageously, the interconnection topology specified in each data structure permits the computational engine to automatically generate these biological models by applying the laws of mass action.

Computational engine 22 includes an equation generation engine for generating symbolic models of biological processes as well as an engine for generating computational models of dynamic biological behavior based upon the symbolic models. The equation generation engine 24 automatically transforms each data structure into at least one system of equations describing a specific biologic process. This system of equations is referred to as a symbolic model. These symbolic models may be stored in the system for later use in modeling the same biologic process, or alternatively, the models may be coupled with other symbolic models generated by the system to model different biologic processes. As discussed in more detail below, any number of symbolic models may be coupled together to produce models of complex

subcellular, cellular, tissue or organ process. In this way, complex models which link functional behavior to subcellular and cellular, as well as system processes may be derived. Equation generation engines 24 such as those which are a part of commercially available software tools such as Mathematica and Maple are well suited to the practice of the present invention.

Computational engine 22 generates a computational model reflective of the biological process defined by the symbolic model. A computational model refers to a software procedure for numerical simulation of the behavior of the symbolic model.

As previously noted, computational models are software procedures for numerical simulation of the behavior of the symbolic model. Typically, the tools used to generate numerical simulations include those available from IMSL (International Mathematical and Statistical Library); NAG (Numerical Algorithm Group); and MATLAB (Mathematical Laboratory); and Visual Numerics and the like.

Optionally, the symbolic models may also be translated into computer code such as Fortran and C++ by conventional means readily available in the prior art. Advantageously, typeset equations expressed in markup languages such as TeX, LaTeX or HTML can be automatically derived from the symbolic models, thereby tremendously simplifying the process of model documentation. Moreover, critical components of computational models, for example, Jacobian matrices that are used by certain numerical integration algorithms can be derived in an automated fashion from the symbolic models.

As previously indicated, equation generation engine 24 automatically generates symbolic models in the form of coupled systems of differential equations from the information contained in the data structures. The models so generated will retain the attributes of every component of the data structures used to generate the model. For example, the attributes Organism:Cell:State:Location:ModelType would contain the attributes "Organism", "Cell", "State", and "Location" as previously discussed, with the

equation that is simpler than the original component model (*a lumped model*). Once the form of the reduced model is selected, parameters of the new model component are adjusted to fit the behavior of the original model component over the range of interest to the user, using regression techniques available in software products such as MATLAB (Mathworks, Nattick, MA), IDL (Research Systems, Boulder, CO) and PV-WAVE (Visual Numerics, Inc., Houston, TX) and in numerical libraries from NAG, Ltd. (Numerical Algorithm Group), Visual Numerics and the like. These packages can also be configured to provide statistical goodness-of-fit estimates that can be used to determine the statistical significance of the resulting simulations. The fitted correlation function or lumped model component is then used in the place of the original when performing computational simulations. When the form of the simplified model is different than that of the original model, a hybrid solver must be used. For example, correlation functions often introduce algebraic constraints to systems of differential equations.

Software systems that simultaneously determine the form of the simplified model and regress the parameters of the model to the original may also be used. These systems often make use of pattern recognition and machine learning algorithms to achieve a high quality approximation with a simplified model. An example is the HDMR (High-Dimensional Model Representation) system of Shorter, Ip and Rabitz.

Alternatively, practical differential equation solver packages use adaptive methods that switch automatically between explicit and implicit time stepping methods, providing marked speed improvements particularly useful for models which exhibit stiff behavior at least at one point in a simulation. Examples of software with adaptive solvers include the ODEPACK family of solvers from the Lawrence Livermore National Laboratory and DASSPK family of solvers by Linda Petzold of the University of Minnesota. These solvers have the ability to handle mixed continuous-discrete time and differential-algebraic systems. They also can take advantage of the natural sparsely of the system of equations, providing even larger performance gains.

When the model consists of a system of partial differential equations (PDE), or coupled differential algebraic systems, parallel algorithms are useful to solve the problems. These multiple-processor codes use industry standard libraries to control algorithm and data flow. Examples of these libraries are the Message Passing Interface (MPI) and the Parallel Virtual Machine (PVM). Both allow a single simulation application to run on heterogeneous machines, and allow each process to work on different tasks. In this way, a heterogeneous problem can run simultaneously on a network consisting of one or more personal computers, workstations and supercomputers.

Thus, in one embodiment of the present invention the symbolic and computational models define the time rate of change of the concentration of reaction intermediates, or of other state variables that effect subcellular, cellular or higher order processes. Consider, for example, the biochemical pathway shown in FIG. 8. Let A, B, C, and D represent elementary data structures defining the pathway wherein "i" or "j" are generic representations for the various states such as A, B, C, or D (K_{ij}, K_{AB} or K_{CA} or ...), and K_{ij} represents the transition rate between states i and j that are defined by the various Frate and Brate pointers. Applying the laws of mass action will yield the following system of ordinary differential equations describing the dynamics of this system.

$$dA/dt = -A(K_{AB} + K_{AC}) + BK_{BA} + CK_{CA}$$
$$dB/dt = AK_{AB} - B(K_{BA}+K_{BC}+K_{BD}) + CK_{CB} + DK_{DB}$$
$$dC/dt = AK_{AC} + BK_{BC} - C(K_{CA}+K_{CB})$$
$$dD/dt = BK_{BD} - DK_{DB}$$

Since these equations are completely defined by knowledge of the connectivity of the network, and knowledge of the various transition rate constants, and since these quantities are all stored in the databases, the equations may be generated automatically on computer. They may also be

integrated in time, or be analyzed using the numerical methods described herein.

As another illustration, consider the hierarchy of cellular metabolism which originates from the level of the gene. A qualitative representation of the actions of genes and their activation or inhibition would be represented, through the standard notation for chemical reactions, as:

$$A + G_1 \rightarrow (A + G_1)^+$$

Or:

$$I + G_1 \rightarrow (I + G_1)^-$$

Subsequently, the synthesis of a protein can be represented as:

$$(A + G_1)^+ + \text{Amino acids} \rightarrow P_1$$

This representation bypasses the process of transcription involving mRNA synthesis, since the product of gene activation or inhibition is finally a protein. For proteins, that are enzymes, an enzymatic reaction is represented as:

$$M_1 + E_1 + \text{cofactor} \rightarrow M_2 + E_1$$

The presence or absence of an inhibitor of the enzyme could also be represented by:

$$E_1 + I \rightarrow (E_1 + I)^-$$

Where, $(E_1 + I)^-$ is the enzyme-inhibitor complex.

This zero order qualitative model can be used to develop more complex structured models. For example, the most detailed BRN model possible is a description of the temporal variance of every species within the cell. A quantity within a cell, M_i, can be involved in several processes that contribute to its net formation and consumption. These processes can be transported across an organelle with the cytoplasm, synthesized or consumed in a chemical reaction and transport across a cell membrane.

The basic mass balance for such a system can be represented by the following mathematical relationship:

$\frac{dm_i}{dt} = \sum_j s_{ij} v_j$, Where, s_{ij} is the stoichiometric coefficient associated with each flux v_j. Each flux v_j, is some function of the metabolite concentrations, i.e., v_j,

This material balance under steady state conditions will reduce to the algebraic relation: $\sum_j s_{ij} v_j = 0$

Or, for all intermediates simultaneously at steady state, the individual balance equations can be rewritten in matrix form,

$$\mathbf{S} \cdot \mathbf{v} = \mathbf{0}$$

Where S is the stoichiometric matrix and v is the vector of metabolic fluxes. This stoichiometric relationship can also be viewed as a connectivity relationship that connects the intermediates through the fluxes that they are involved in. The stoichiometric relationship can be used in identifying the properties of a network of metabolic reactions. These properties include, the identification of conserved quantities, and pathways of fundamental importance in the connectivity of a network.

These models of intracellular reactions would be integrated into systems models by the following mathematical representation:

$\frac{dm_i}{dt} = \sum_j s_{ij} v_j$, describes the change of metabolites, within a single cell.

If, x_i, is used to represent whole cells, the change of cell populations can be described as:

$\frac{dx_i}{dt} = r_i^f - r_i^d$, where r_i^f, is the rate of formation of the cell species, x_i, and r_i^d, is the rate of death of the cell species. Each rate is a complex function of metabolites and cells, i.e.,

$$r_i^f = r_i^f(x_i, m_i)$$
$$r_i^d = r_i^d(x_i, m_i)$$

Since the concentrations of metabolites, that are secreted or removed by cells, is influenced, by the concerted action of many cells, these metabolites are represented by

$$\frac{dm_i^e}{dt} = \left(\sum_j s_{ij} v_j^e \right) x_i ,$$

where the influence of the metabolite concentration by the overall cell population is factored into the equation describing the intracellular reactions. Thus a mathematically complete description of a system of cells can be described as,

$$\frac{dx_1}{dt} = r_i^f - r_i^d$$

$$\frac{dm_i^e}{dt} = \left(\sum_j s_{ij} v_j^e \right) x_i$$

$$\frac{dm_i i}{dt} = \left(\sum_j s_{ij} v_j^i \right) x_i$$

Where, m_i^e, represents external metabolite concentrations, and m_i^i, internal metabolite concentrations.

Advantageously, the macroscopic characteristics of a cell population and a single cell description are completely described in such a mathematical formalism.

The models generated by the system may be further transformed into textual or graphical representations by use of graphical user interface 23.

Optionally, the models may also be analyzed using techniques from nonlinear systems theory. For example, public domain tools such as AUTO and XPP, accessible from the Internet can be used to perform analyses of the parameter dependence and asymptotic behaviors of biological models. This permits the calculation of qualitative behaviors of complex models as key model parameters are changed.

Graphical User Interface

Graphical user interface 23 provides a user with input to and output from information in the system. More specifically, graphical user interface 23 may be used to (1) draw genetic and biochemical pathway diagrams, and to enter functions specifying rate constants in these reaction pathways, for storage in database 11 or for symbolic and computational modeling; (2) interconnect EDS, BDS, and PDS data structures in order to compose hierarchical models of biological systems; (3) construct and manipulate biophysical and structural models; (4) display and interact with previously developed genetic, biochemical, biophysical, and structural models; and (5) control formulation and solution of computational and symbolic models, and to view simulation output.

Graphical user interface 23 can be customized for a particular application. Typically, interface elements such as video monitors, touchscreens, keyboards, a mouse, printers and the like may be used.

Creation of a Model

In accordance with the present invention, a model may be created to study any type of subcellular, cellular, tissue or organ information as, for example, the function of a gene, a specific biological process, the behavior of a target protein in the presence of a particular drug, or system functions in response to certain therapies. Based on the problem to be solved, the user will select the information from the database that will serve as the building blocks for developing the model. For example, a user may wish to predict the quantity of certain intermediates in the pyruvate dehydrogenate reaction in a specific cell type both in health and disease. In this instance, a model would be generated based upon the structural elements of the cell together with the biochemical and biophysical processes and their associated interconnection topologies.

These models can be displayed on the display monitor. In general, the user will be presented with a palette of icons that can be browsed, where each icon represents some binary or pathway data structure, such as a

biochemical or biophysical mechanism previously defined and stored in the system. The user would interact with this graphical display by use of a mouse. The user can add these components to the structural model by selecting icons and dragging them to the point of insertion in the model.

The user may view information regarding the biochemical/ biophysical mechanism inserted into the model by clicking on the representation of that mechanism. For example, clicking on the icon for the pyruvate dehydrogenase reaction will trigger a display of the pathway illustrated in FIG. 2 on the display monitor. The user can then query the system for information associated with the intermediates of these reactions. Clicking on, for example, pyruvate dehydrogenase will initiate a pop-up display of all of the attributes describing pyruvate dehydrogenase that may be examined. The user will select from one of these attributes. Advantageously, the linked attribute list will cause the system to initiate a query and display of information to the appropriate database, for example, a display of the gene sequence of pyruvate dehydrogenase. All of the elements of the attribute list associated with pyruvate dehydrogenase could be displayed in this manner. Thus, the simple act of clicking on pyruvate dehydrogenase retrieves for the user all information on pyruvate dehydrogenase stored in the system and makes it available to facilitate modeling. This configuration permits a user to interact with graphical user interface 23 to retrieve any of the information associated with or generated by the system. In this way, the user is presented with a complete representation of specific biological processes.

If desired, the user can invoke an equation generation engine to generate a symbolic set of coupled differential equations defining the model. These equations could be saved as part of a documentation of the model and/or they may be input into translators that would map them into computer instructions in the desired programming language. This source code can then be linked with a computational engine to produce executable code for modeling the cell. Preferably, this executable code may be stored in the system for future use.

In another example, the user may wish to model intracellular protein in trafficking which occurs following ligand:receptor interactions which occur in signaling processes that allow molecules to move from the plasma membrane, or the cytosol, to the nucleus. For example, in T-cell signaling, the T-cell receptor binds a ligand (MHC and antigen) to initiate a signaling cascade that progresses through the cytosol and culminates in both new protein synthesis and in active inhibition of gene activity. Creation of a single-cell model in accordance with the present invention will allow a user to follow protein signaling events, and in this way, define possible gene(s) and gene modulation activity of the protein in question.

Moreover, while the intracellular representation of molecules (and their functional moieties) are in some cases unique to one cell type (i.e., the expression of the CD4 molecule is restricted to CD4 T-cells), this is not true for many intracellular molecules. Accordingly, the results of a single intracellular model may apply to a number of other cells in an organism. Thus, the protein signaling system described above may have broader implications in cellular signaling in other cells of the organism. For instance, a CD4 T-cell secretes IL-4, Il-5 which are cytokines that affect the performance of the B-cell, which is another component of the immune organ system. The T-cell also has specific molecules, e.g. CD40 ligand which binds to a CD-40 receptor on a macrophage. Thus there are processes from a T-cell that affect other cell types within the organ. The cell models can therefore be combined by linking the BRNs in the T-cell that form IL-4 to the BRNs that IL-4 affects in the B-cells, and by the BRNs that form CD40 ligand to the BRNs affected by the CD40 ligand-receptor compex in the macrophage.

These linked models constitute a model of an organ system, which is applicable to various clinical and pharmaceutical purposes. Drug development focuses on targeting specific reactions and molecules in a cell. Since the organ model is built from several cell models that are built from several BRNs, a single step or a number of single steps can be removed or changed in the model to mimic the effect of a drug. So one or a few steps in synthesis of IL-4 in the T-cell can be targeted in the model simulator and its

characteristics can be changed. The overall results on the organ function can be measured by tracking the effect of these changes on the function of all the cells and tissue types and the overall organ function. Specifically, the effect on the B-cell function and the effect on macrophage function can be tracked. The ability to respond to an infection can also be tracked, which is a feature of organ function. In a clinical trial, the changes to Il-4 production can be changed to look at the organ function change. In clinical diagnosis, in a disease such as rheumatoid arthritis, a patient's characteristics can be input into the model and then measured against a normal person's model, to obtain the specific abnormalities at the cell level for that patient.

Validation of Models

The models generated in accordance with the present invention are validated against information gleaned from clinical data, expert opinion, or a combination thereof. Where disagreement between the model and known data exist, the model is corrected iteratively until a correlation is found. After the model is created, the system compares the solution of equations to experimental data, measuring goodness-of-fit of the model. A user can interactively adjust any of the attributes associated with the model to create a new hierarchical description which approximates user selected properties of the experimental data. In this instance, a system identification engine can be invoked to adjust the parameters of the equations defining the model to create a new system of equations, the solution of which approximates user selected properties of the experimental data. The system identification engine includes routines for optimally updating the parameters of a model, taking into account measurement and model uncertainty. Example algorithms include Kalman Filters and batch least-square filters. The system identification engine can also include algorithms for estimating the quality of the fit of the model to the experimental data. Complete systems for doing system identification are available as add-on packages to Matlab (Mathworks, Nattick, MA), and integrated in the Scilab data analysis system (INRIA, France).

Linking Models

Several models may be linked together. For example, a number of different biochemical or biophysical mechanisms may be inserted into a single structural model. In this instance, several models would be merged into a single model by an interface which would effectuate the flow of information between the respective models. For example, the outputs or intermediates in a biochemical reaction network describing a PDS such as described in FIG. 2, may act directly or indirectly to modulate the function of another process such as the BDS representing an ion channel model of FIG. 5. A specific case may be the output variable of adenosine triphosphate (ATP) of glycolytic biochemical reaction networks and its modulating action of ATP-sensitive membrane potassium channels.

Single cell models may be integrated with organ models. For example, intracellular models of cell states for normal and diseased states can be generated in order to allow cell types, and mediators of cellular function to be modulated and analyzed in a specific disease state. Such information can be used to identify specific points of disease progression best suited for therapeutic intervention.

By way of illustration, inherent in an immune cell/organ integrated model are network regulation dynamics, some of which are universal (i.e., mass-balance and metabolism) and some of which are unique to the immune system (i.e., differentiation). Single cell models that could be generated in this instance include macrophages, dendritic cells, naive T-cells (CD4, CD8), effect on and memory T-cells, B-cells, plasma cells, mast cells and basophils. These models could be integrated into an organ model in order to provide a more complex representation of a biological system.

As another example, a model for therapeutic intervention of rheumatoid arthritis could be developed based on animal models of arthritis induced with antigens or infectious agents. In these models, disease severity correlates with a dominant Th1-type cell response characterized by a higher ratio of IFN-γ to IL-4. It is known that Th2 cytokine therapy (e.g., infusion of IL-4) may suppress disease symptomatology. It is also known that IL-1,

IL-6 and TNF-∝ are secreted in very high levels in arthritic joints and therapies directed to these mediators may be effective. In this instance, an intracellular model of the TNF-∝ could be generated in health and in various states of disease progression. Against these single cells models, anti-TNF-∝ reagents may be screened in order to ascertain suitable points for therapeutic intervention. FIG. 11 illustrates the information obtained from these modulators that may be used in the creation of a model of Th1 cell differentiation in rheumatoid arthritis.

As still another example, consider asthma, a complex inflammatory disease with many cell types and cytokines participating in the generation of late-phase inflammation. Prior to the present invention, an understanding of which all types are important sources of these cytokines was limited due to the inability to directly compare the relative contribution of individual cell populations. It was known, however, that Th2 responses which contribute to airway eosinophia, mucus production and IgE synthesis are key features of asthma. Intracellular modulation of transcription factor GATA-3, which regulates the expression of cytokines IL-4, IL-5 and IL-13, which are secreted by Th2 cells, but not Th1 cells, at various stages of disease progression could be studied in order to develop GATA-3 as a potential therapeutic target in the treatment of asthma. The information obtained from these models can be incorporated into a multicellular model of Th1/Th2 cells to ascertain the effect of cytokine expression on skewing Th1/Th2 balance towards a Th2-type cell and the rate of GATA=3 in this system. As is illustrated in FIGS. 12a and 12b, a much greater level of cytokine production is present in T-cell differention to Th2-tpye cells rather than to Th1-type cells.

Display of Model Results

Output data from each simulation, as well as the underlying data, may be displayed on the graphical user interface. Output data may include gene data (i.e., recruitment, activator and expression), in expression data (i.e., activator and expression), protein modulation data (i.e., phosphorylation, glycosylation, association, etc...) cell turnover rates (i.e., recruitment, proliferation, differentiation, death), protein accumulation, calcium fluxes, cell trafficking rates, uniquely defined parameters of clinical relevance to track pathophysiology and the like. FIG. 13 provides an example of a descriptive report generated in response to a specific modeling query. FIG. 14 provides an illustrative graphical model output for the dynamic change in concentrations or levels in a T-cell that is characteristic of the behavior of that cell, and is characteristic of the signaling within the T-cell. A user can modify the data from each simulation as well as the underlying information which the data represents. The user may also customize the physical appearance of the graphics or textual appearance of the output data. By way of illustration, the user can double-click on a compartment of the model, and would be presented with a list of variables used. The user could select a variable and display that variable on a graph drawn in a separate window. Optionally, the user could modify the underlying variable and generate a new model. Alternatively, the user could select "global" variables, that is, those state variables defined everywhere within a model and display the global variable using a color coding scheme over the entire model domain.

Model Uses

The model can be used to store and search all existing biological information (i.e., genetic, biochemical, biophysical and anatomical) on a given biological process at the subcellular, cellular or multicellular level. As such, the model may be used to integrate knowledge across all biological systems.

The model thus provides a means for collecting and synthesizing biological information into a format by which function within a biological system may be analyzed. For example, the function of a particular gene could be ascertained by invoking the model to determine the sequence of the gene of interest and identify homologous genes and BRN's in which the homologous gene participates. Based on the BRN'S, the dynamic behavior of the homologous genes could be modeled, providing quantitative insight into the possible functional role of the gene of interest. Thus, the model could provide not only homology searches based on linear sequence analysis, but also functional search capabilities based on the similarity of the BRN's in which a gene participates.

In addition, the model may be used in drug discovery, as for example, to analyze the behavior of molecular targets in the presence of a particular drug. Computational models of drug/gene action would be generated and incorporated into models of physiological function in accordance with the present invention. These multi-dimensional models could then be used to screen candidate compounds.

Computer System

The present invention may be implemented on any computer architecture in any configuration such as multi-tiered or clustered services or a client-server paradigm. Certainly, the type of computer system will depend on the complexity of the model(s) and the choice of an appropriate system is readily available to a skilled artisan. Typically, the components of such a computer system would include a central processing unit, RAM, ROM, I/O Adapter, data storage space, a graphical user interface having a keyboard, mouse and speakers attached thereto as well as an operating system and software capable of providing Internet connectivity.

The following examples are presented to provide a more complete understanding of the invention. The specific techniques, conditions, materials, proportions and reported data set forth to illustrate the principles

and practice of the invention are exemplary and should not be construed as limiting the scope of the invention.

Example 1

This is an example of a "CellML" description of the basic FitzHugh-Nagumo model generated in accordance with the present invention. (CellML is a subset of XML that is used to describe a cell model or a series of cell models.) For purposes of this model it is treated as an ion current. This model contains two differential equations:

$$du/(dt = (u - u^3/1 - v) / e \text{ and } dv/dt = eu * (u + b - gv)$$

Where b, g, and e are treated as constants.

```
<CELLMODEL>
 <VEPBOSENAME>Simple Example of a cell model with a single FitzHugh-Nagumo element</VERBOSENAME>
 <NAME>FitzHugh-Nagumo Cell</NAME>

A <DRAW> tag is used by the program to describe how the object is represented visually in the cell model.-->

<Draw>
 <DRAWSIZE>8000,8000</DRAWSIZE>
 <POSITION>1000,1000</POSITION>
 <BACKCOLOR>65280</BACKCOLOR>
 <EDGECOLOR>255</EDGECOLOR>
 <DRAW>

The ENVIRONMENT tag is used to define all of the components (chemical species, variables, etc.) within the scope of an element.-->
```

<ENVIRONMENT>

CONSTANT tags are used to contain information about the value of parameters used in this model.

<CONSTANT>
<NAME>b<NAME>
<VALUE>1.0</VALUE>
<CONSTANT>

<CONSTANT>
<NAME>e</NAME>
<VALUE>0.04<VALUE>
</CONSTANT>

<CONSTANT>
<NAME>g</NAME>
<VALUE>0.5</VALUE>
</CONSTANT>

VARIABLE tags are similar to CONSTANT tags except that the values can change during the execution of the model. The values given here represent the initial value for the variable.

<VARIABLE>
<NAME>t</NAME>
<VALUE>0.0</VALUE>
<VARIABLE>

<VARIABLE>

```
        <NAME>u<NAME>
      <VALUE>0.0</VALUE>
    </VARIABLE>

    <VARIABLE>
          <NAME>v</NAME>
       <VALUE>0.0</VALUE>
     </VARIABLE>

    </ENVIRONMENT>
```

IONCURRENT is use to contain the actual model.

```
    <IONCURRENT>

    <NAME>Ifn</NAME>
    <VERBOSENAME>FitzHugh Nagumo Current</VERBOSENAME>
    <DRAW>
      <DRAWSIZE>1000,1000</DRAWSIZE>
      <POSITION>6000,6000</POSITION>
      <BACKCOLOR>32639</BACKCOLOR>
      <EDGECOLOR>8323199</EDGECOLOR>
     <DRAW>
```

The equation for du/dt. The <DERIVATIVE> tag is used to indicate that this needs to be processed as a differential equation.

```
    <DERIVATIVE>
    <reln>
     <eq/>
     <apply>
```

```
<diff/>
<ci>u</ci>
<bvar>
  <ci>t</ci>
 </bvar>
</applv>
</apply>
 <divide/>
  <mfence>
  <apply>
   <minus/>
   <apply>
    <minus/>
    <ci>u</ci>
      <apply>
     <divide/>
     <apply>
      <power/>
      <ci>u</ci>
      <cn>3</cn>
    </apply>
    <cn>3</cn>
   </apply>
  </apply>
  <ci>v</ci>
 </apply>
</mfence>
<ci>e</ci>
</apply>
</reln>
</DERIVATIVE>
```

The equation for dv/dt.

```
<DERIVATIVE>
<reln>
 <eq/>
 <apply>
  <diff/>
  <ci>v</ci>
  <bvar>
   <ci>t</ci>
  </bvar>
 </apply>
 <apply>
  <times/>
  <ci>e</ci>
  <mfence>
   <apply>
    <minus/>
    <apply>
     <plus/>
     <ci>u</ci>
     <ci>b</ci>
    </applv>
    <apply>
     <times/>
     <ci>g</ci>
     <ci>v</ci>
    </apply>
   </applv>
  </mfence>
```

```
</apply>
</reln>
</DERIVATIVE>
</IONCURRENT>
</CELLMODEL>
```

Example 2

This example describes the CellML tags used by the present invention to represent a cell model.

CellML uses MathML to model the actual equations that it references.

The tags in CellML are designed to be hierarchical in nature; that is a given tag is generally used to describe the properties of its parent. For example, a <SIZE> tag can be used to indicate the size of a <CELLMODEL>. When the CellML code is read by the present invention, a series of "objects" (i.e. Class objects in C++ or Java parlance) is created that has close to a one-to-one correspondence with the original source code.

CellML tags are broken down into several distinct classes, based on their purpose:

- ***Basic Elements*** are tags that are used to describe a general property such as the name of an object or its size. These are the lowest level elements and can be used by several different kinds of tags.
- ***General Cell Model Elements*** are used to represent the general properties of a cell and the biochemical processes that are being modeled.
- ***Specific Cell Model Elements*** are similar to "General Cell Model Elements" except that they are used to represent a higher level of abstraction.

- ***Drawing Elements*** are used to supply information on how a Cell Model is to be displayed visually, and how it interacts with the GUI.

The contents of each CellML document will obey a set of grammar rules defined in the CellML Document Type Definition (DTD).

TYPE	TAG	DESCRIPTION	SUB-TAG
Basic Elements	NAME	The short name of an object	---
	VERBOSENAME	A longer name for the object	---
	VALUE	Tag used to store a single numeric value	---
	CONSTANT	Used to define a fixed parameter	NAME VALUE UNITS
	VARIABLE	Used to represent a single state variable. This contains both a value at the current point in time, and at the initial condition.	NAME VAULE UNITS HISTORY
	UNITS	The units for a VALUE (e.g., [mm], [g/mol], etc.)	---
	EQUATION	Used to contain a single MathML equation	RELN (MathML code)
	POSITION	The physical position of an object in its parent object. Can be used to define 3D (x,y,z), 2D (x,y) or 1D (x) position.	---
	SIZE	The physical size of an object. Can be 3D, 2D, or 1D.	---
	DBLINK	Database Linkage. Used to hold a pointer to information on an element in a database.	---
General Cell Model Elements	MODEL	The highest level object, consisting of one or more CELLMODELS.	CELLMODEL
	CELLMODEL	A single unit in a model. This tag may contain information about its location relative to other CELLMODELS.	PATHWAY REACTION COMPONENT IONCURRENT REFERENCE
	PATHWAY	Tag that describes a set of reactions, for example where: Reactant ⇐ ⇒ Product in	REACTION

TYPE	TAG	DESCRIPTION	SUB-TAG
		multiple steps. (PDS)	
	REACTION	Describes a single elementary reaction. Reactants ⇐ ⇒ Products with Forward and Reverse kinetics. (BDS)	COMPONENT KF KR
	ENVIRONMENT	Encapsulates all of the components and properties of a CellModel.	COMPONENT CONSTANT
	COMPONENT	Representation of a single chemical species. This tag can contain information on concentration, formula, and structure. (EDS)	VARIABLE DBLINK
	HISTORY	The value of a "property" (e.g., COMPONENT, VARIABLE) as a function of time.	---
	KF	Forward Reaction kinetics for single REACTION.	COMPONENT EQUATION
	KR	Reverse Reaction kinetics for single REACTION.	COMPONENT EQUATION
	INTEGRATE	Used to store information about the type of integration to be run. Contains starting and stopping time, time step, and type of integrator to be used.	---
	PROTOCOL	Description of a time based protocol applied to a Cell Model.	VARIABLE
	REFERENCE	A bibliographic tag used to describe where this model came from. This will ultimately contain several sub-tags for elements such as "<author>", "<volume>", "<date>", etc.	---
Specific Cell Model Elements	IONCURRENT	Used to represent an ion current.	GATE COMPONENT
	GATE	A Hodgking-Huxley type gate element.	EQUATION
	PROTEIN	Descriptions of a gene product.	---
	DRUG	Description of drug effect on elementary, binary or pathway data structures or protein or variable.	---

TYPE	TAG	DESCRIPTION	SUB-TAG
Drawing Elements	DRAW	Tag encapsulating information needed to draw an object in a window.	SIZE POSITION FORECOLOR BACKCOLOR EDGECOLOR
	LOGCOORD	Tag containing information on transforming from physical to logical coordinate system. This can also control the rendering of a 3D object on a 2D screen.	---
	FORECOLOR	The foreground color of an object.	---
	BACKCOLOR	The background color of an object.	---
	EDGECOLOR	The color of an object's edge.	---
	POSITION	The position of an element in logical drawing space.	---
	DRAWSIZE	The size of an element in logical drawing space.	---

Example 3

Component	Description
Class Library	The C++ class objects that are used to create a cell model and simulation, and describe its mathematics and chemistry.
CellML Definition / DTD	A definition of the mark-up language used to describe the models of the present invention. This involves developing the set of tags to use with CellML and putting together a DTD to formalize the syntax and allow models to be validated by browsers.
Parser	The Parser is used to generate "run-time objects" of the cell components based on an XML input file and the system class library data. This consists of two components: (1) The raw XML parser that reads the input files and generates the hierarchical tag and text nodes. (2) The "object constructor" which creates and initializes objects based on the XML content.
Class Converter	Conversion of XML tags to leaner MathML class objects.
Computation Engines	Systems that are used to integrate cell model over time and evaluate reactions.

Component	Description
Component Editor	A form-based GUI that is used to create and initialize the set of chemical components within an "environment" of a cell model.
Reaction Editor	A form-based GUI used to graphically create a chemical reaction or pathway.
Equation Editor	Used to allow mathematical equations to be entered into modes in an algebraic format (as opposed to the native MathML format).
Database Linkage	Used to connect the system of the present invention to external database system containing information on cell components.
Visual Editor	Allows the user to graphically edit a cell model using features such as drag-and-drop and in-lace activation.
Data Plotting System	A generic 2D and possible 3D plotting system. This is a full-featured system giving complete control over the layout, scaling, and visual format of a plot.
Dynamic Form System	This system is used to create a dialog form from an XML input file or a system model object. This allows cell models to be edited and manipulated in a very flexible way.
Object Serialization	Used to read and write (serialize) object-based cell models in a binary format. This is required to enable releasing proprietary cell models.
Output Engines	The output engines are used to take an object based cell model and generate text output in several different formats. Formats being considered include: (1) XML output file (2) Fortran and/or C equations defining the cell model behavior (3) Some form of visual presentation of the mathematical equations (HTML/MathML, TeX, rich-text).
Java /Web Model Viewer	A tool that allows CellML models to be viewed in a browser.
Java-based Model Editor	A system editor designed to run in a Java environment.
User Documentation	User manual describing the use and operation of the system of the present invention.
Online Help System	Online version of user manual and integration of this into the system of the present invention.

Example 4

One of the unique aspects of the present invention is the ability of the system to build models with hidden mathematics. This allows users to

construct complex models of biological systems without in-depth knowledge of mathematical modeling.

FIG. 15 represents a graphical model of the various reaction pathways present when T-cells activate. Consider the initial conditions for the components of the T-cell model set for the below:

Initial Conditions for the Components of the T-Cell Model

STAT6p = 1.0
GATA3 = 1.0
cmaf = 1.0
Y = 1.0
NFIL6 = 1.0
X = 1.0
IL4 = 0.0
IL5 = 0.0
IL13 = 0.0
IL6R = 0.0
STAT6 = 3.0
NFATcP = 5.0
NFATc = 1.0
IL4R = 0.0
Jak3 = 3.0
NFkb = 1.0
ras = 1.0
raf = 1.0
rac = 1.0
p38Jun = 1.0
JunFos = 1.0
TCR = 1.0
LATp = 1.0
PIP2 = 1.0
IP3 = 1.0
DAG = 1.0
PLCg = 1.0
PI3K = 1.0
Gefs = 1.0
SLPvav = 1.0
Ca2 = 2.0
PKC = 1.0
Calcineurin = 1.0
PI = 5.0
CD28 = 0.0
IKK = 1.0
Z = 5.0
Fos = 1.0

STAT4 = 3.0
STAT4p = 1.0
IFNg = 0.0

The following system of equations represent T-cell activation for the initial component conditions listed above:

Equations for representing Components

dSTAT6p/dt = k7 * STAT6 + -k0 * STAT6p
dGATA3/dt = k0 * STAT6p + k26 * Z + -k3 * GATA3
dcmaf/dt = k0 * STAT6p + -k5 * cmaf
dY/dt = k0 * STAT6p + -k2 * Y
dNFIL6/dt = k0 * STAT6p + k6 * IL6R + k19 * LATp + -k4 * NFIL6
dX/dt = k3 * GATA3 + -k1 * X
dIL4/dt = k1 * X + k2 * Y + k4 * NFIL6 + k5 * cmaf + k9 * NFATc + k16 * JunFos
dIL5/dt = k3 * GATA3 + k11 * NFkb + k12 * NFkb + k16 * JunFos
dIL13/dt = k3 * GATA3 + k12 * NFkb + k16 * JunFos
dIL6R/dt = -k6 * IL6R
dSTAT6/dt = -k7 * STAT6
dNFATcP/dt = -k8 * NFATcP
dNFATc/dt = k8 * NFATcP + k28 * raf + -k9 * NFATc
dIL4R/dt = -k10 * IL4R
dJak3/dt = k10 * IL4R
dNFkb/dt = -k11 * NFkb + -k12 * NFkb
dras/dt = k25 * Gefs + k30 * DAG + -k13 * ras
draf/dt = k13 * ras + k27 * PKC + -k28 * raf
drac/dt = k24 * SLPvav + -k14 * rac
dp38Jun/dt = k14 * rac + k28 * raf + -k15 * p38Jun
dJunFos/dt = k15 * p38Jun + k29 * Fos + -k16 * JunFos
dTCR/dt = -k17 * TCR
dLATp/dt = k17 * TCR + -k19 * LATp
dPIP2/dt = k22 * PI + -k18 * PIP2
dIP3/dt = k18 * PIP2 + -k21 * IP3
dDAG/dt = k18 * PIP2 + -k30 * DAG
dPLCg/dt = k19 * LATp
dPI3K/dt = k19 * LATp + k23 * CD28
dGefs/dt = k19 * LATp + -k25 * Gefs
dSLPvav/dt = k19 * LATp + -k24 * SLPvav
dCa2/dt = k21 * IP3 + -k20 * Ca2
dPKC/dt = k20 * Ca2 + k30 * DAG + -k27 * PKC
dCalcineurin/dt = k20 * Ca2
dPI/dt = -k22 * PI
dCD28/dt = -k23 * CD28
dIKK/dt = k23 * CD28
dZ/dt = -k26 * Z

dFos/dt = k28 * raf + -k29 * Fos
dSTAT4/dt = -k31 * STAT4
dSTAT4p/dt = k31 * STAT4 + -k32 * STAT4p
dIFNg/dt = k32 * STAT4p

Referring back to FIG. 15, a user could click on the "." linking each of the model components and insert various kinetic parameters (accessible from the database) thereby altering the system of equations representing the model. In this way, the model incorporates qualitative simulators with quantitative methods.

This model can be integrated into a system model, such as T-cell differentiation in rheumatoid arthritis illustrated in FIG. 10. This allows the user to simulate the heterogeneous time scales found in the system model via qualitative and quantitative analysis.

Having thus described the invention in rather full detail, it will be understood that such detail need not be strictly adhered to but that various changes and modifications may suggest themselves to one skilled in the art, all falling within the scope of the present invention as defined by subjoined claims.

CLAIMS

What is claimed is:

1. An interactive system for mathematically modeling biological information from the subcellular to the cellular, tissue, and organ level, comprising:

a) at least one database containing biological information which is used to generate at least one data structure having at least one attribute associated therewith;

b) a user interface for interactively viewing and editing attributes the data structure to create at least one hierarchical description of subcellular, cellular, tissue or organ function;

c) an equation generation engine operative to generate at least one mathematical equation from at least one hierarchical description; and

d) a computational engine operative on at least one mathematical equation to model dynamic biological behavior.

2. An interactive computer-implemented system as recited in claim 1, wherein the user interface allows for the linking together attributes from a plurality of data structures.

3. An interactive computer-implemented system as recited in claim 1, wherein the data structure is selected from the group consisting of elementary, binary or pathway data structures or a combination thereof.

4. An interactive computer-implemented system as recited in claim 3, wherein the binary and pathway data structures are arranged as state transition diagrams.

5. An interactive computer-implemented system as recited in claim 1, wherein the database comprises at least one external database.

6. An interactive computer-implemented system as recited in claim 1, wherein the mathematical equation comprises at least two equations.

7. An interactive computer-implemented system as recited in claim 6, wherein the equations represent linked attributes derived from the plurality of data structures.

8. An interactive computer-implemented system as recited in claim 6, further comprising a correlation engine for solving the equations generated by the system.

9. An interactive computer-implemented system as recited in claim 1, wherein the data structure comprises an elementary data structure having at least one of a variable or protein.

10. An interactive computer-implemented system as recited in claim 1, wherein the data structure comprises a binary data structure which is a composition of at least two elementary data structures having at least one transition therebetween.

11. An interactive computer-implemented system as recited in claim 1, wherein the data structure comprises a binary data structure which is a composition of at least two elementary data structures having at least one rate constant associated therewith.

12. An interactive computer-implemented system as recited in claim 1, wherein the data structure comprises a pathway data structure which is a composition of more than one binary data structure.

13. An interactive computer-implemented system for mathematically modeling biological information from the subcellular to the cellular to the system level comprising:

a) at least one database containing biological information which is used to generate a plurality of data structures, each having at least one attribute associated therewith;

b) a user interface for viewing, editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system;

c) an equation generation engine operative to generate a plurality of mathematical equations from at least one hierarchical description of a biological system; and

d) a computational engine operative on the plurality of mathematical equations to model dynamic biological behavior.

14. An interactive computer-implemented system as recited in claim 13, wherein the mathematical equation comprises at least two equations.

15. An interactive computer-implemented system as recited in claim 13, wherein the equations represent linked attributes derived from the plurality of data structures.

16. An interactive computer-implemented system as recited in claim 13, wherein the plurality of mathematical equations approximate a simplified system of a specified function or a lumped model.

17. An interactive computer-implemented system as recited in claim 13, further comprising a correlation engine operative to generate a simplified system of equations.

18. An interactive computer-implemented system as recited in claim 13, further comprising explicit and implicit means for numerically solving the plurality of mathematical equations.

19. An interactive computer-implemented system as recited in claim 13, wherein the plurality of mathematical equations are solved by parallel algorithms.

20. An interactive computer-implemented system for modeling biological information that accounts for multiple time frames inherent in biological processes comprising:

a) at least one database containing biological information which is used to generate a plurality of data structures each having at least one attribute associated therewith;

b) a user interface for viewing, editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system;

c) a correlation engine operative on at least one hierarchical description of a biological system to generate a simplified system of equations; and

d) a computational engine operative to solve the simplified system of equations to create a model of a dynamic biological process.

21. A method for creating a model of biological information for use with a computer system, comprising:

a) accessing at least one database containing biological information;

b) generating a plurality of data structures, each having at least one attribute associated therewith;

c) interactively viewing editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system; and

d) utilizing a at least one computational engine to mathematically generate at least one model of a biological system reflective of the multiple time frames inherent in biological processes.

22. A method for creating a model of biological information for use with a computer system as recited in claim 21, wherein the database containing biological information described data obtained from at least one laboratory experiment.

23. A method for creating a model of biological information for use with a computer system as recited in claim 21, further comprising interactively viewing heterogeneous outputs generated by the computational engine.

24. A method for linking models of subcellular and cellular processes to systems processes comprising:

a) generating at least one hierarchical description of subcellular function from at least one database containing biological information, the hierarchical description generated from at least one data structure having at least one attribute associated therewith;

b) generating at least one hierarchical description of cellular function by linking a plurality of attributes of subcellular function from the hierarchical description of subcellular function;

c) generating at least one hierarchical description of system function by linking a plurality of attributes of cellular function from the hierarchical description of cellular function; and

d) utilizing at least one computational engine to mathematically generate at least one model of a biological system reflective of a biological system.

25. A method for linking models of subcellular and cellular processes to systems processes as recited by claim 24, further comprising the

step of utilizing at least one computational engine to mathematically generate a model of a biological process after the step of generating at least one hierarchical description of subcellular function.

26. A method for linking models of subcellular and cellular processes to systems processes as recited by claim 24, further comprising the step of utilizing at least one computational engine to mathematically generate a model of a biological process after the step of generating at least one hierarchical description of cellular function.

27. A method for use in drug development comprising

a) accessing at least one database containing biological information;

b) generating a plurality of data structures, each having at least one attribute associated therewith;

c) interactively viewing editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system; and

d) utilizing at least one computational engine to mathematically generate at least one model of a biological system reflective of the multiple time frame inherent in biological processes.

28. A method for use in clinical trials comprising:

a) accessing at least one database containing biological information;

b) generating a plurality of data structures, each having at least one attribute associated therewith;

c) interactively viewing editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system; and

d) utilizing a at least one computational engine to mathematically generate at least one model of a biological system reflective of the multiple time frame inherent in biological processes.

29. A method for use in effectuating clinical diagnoses comprising:

a) accessing at least one database containing biological information;

b) generating a plurality of data structures, each having at least one attribute associated therewith;

c) interactively viewing editing or linking the plurality of data structures to generate at least one hierarchical description of a biological system; and

d) utilizing a at least one computational engine to mathematically generate at least one model of a biological system reflective of the multiple time frame inherent in biological processes.

FIG. 1

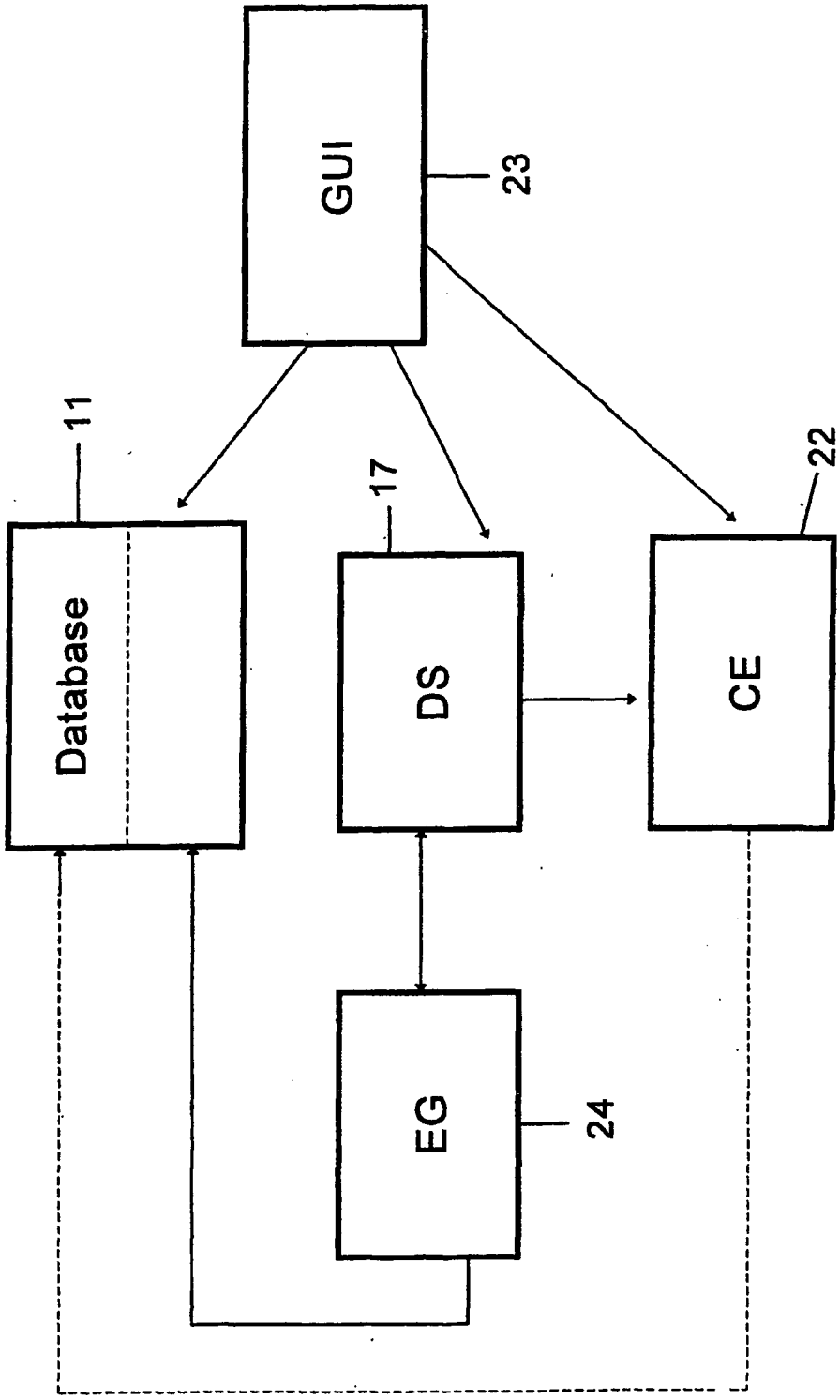


FIG. 2

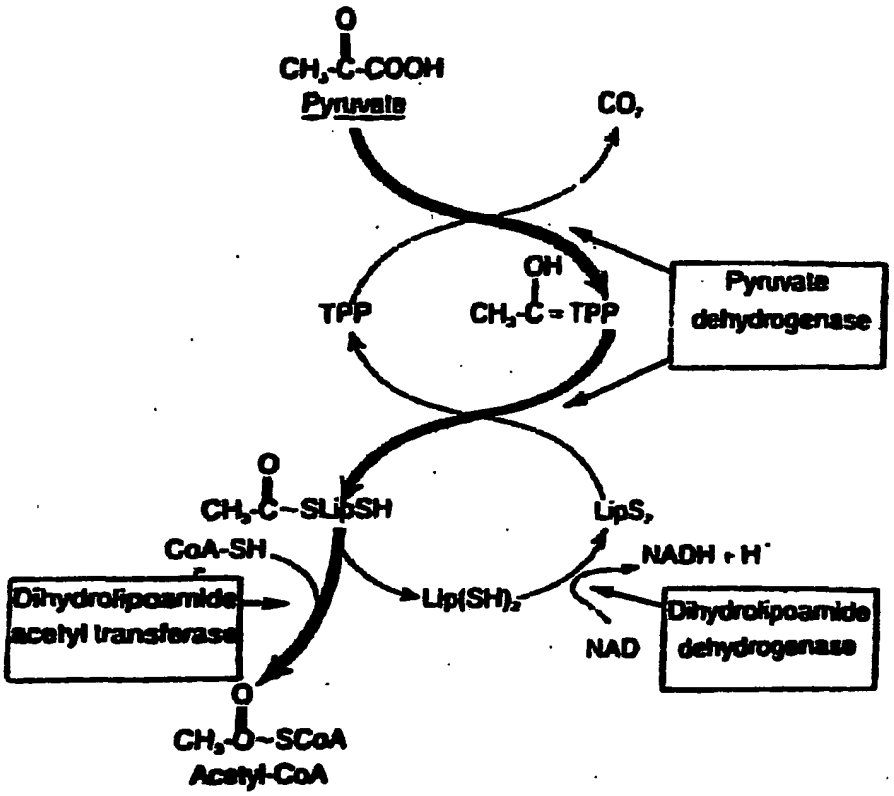

O
CH3-C-COOH
Pyruvate
CO2
OH
CH3-C=TPP
TPP
Pyruvate dehydrogenase
O
CH3-C~SLipSH
LipS2
CoA-SH
NADH + H+
Dihydrolipoamide acetyl transferase
Lip(SH)2
Dihydrolipoamide dehydrogenase
NAD
O
CH3-O~SCoA
Acetyl-CoA

FIG. 3

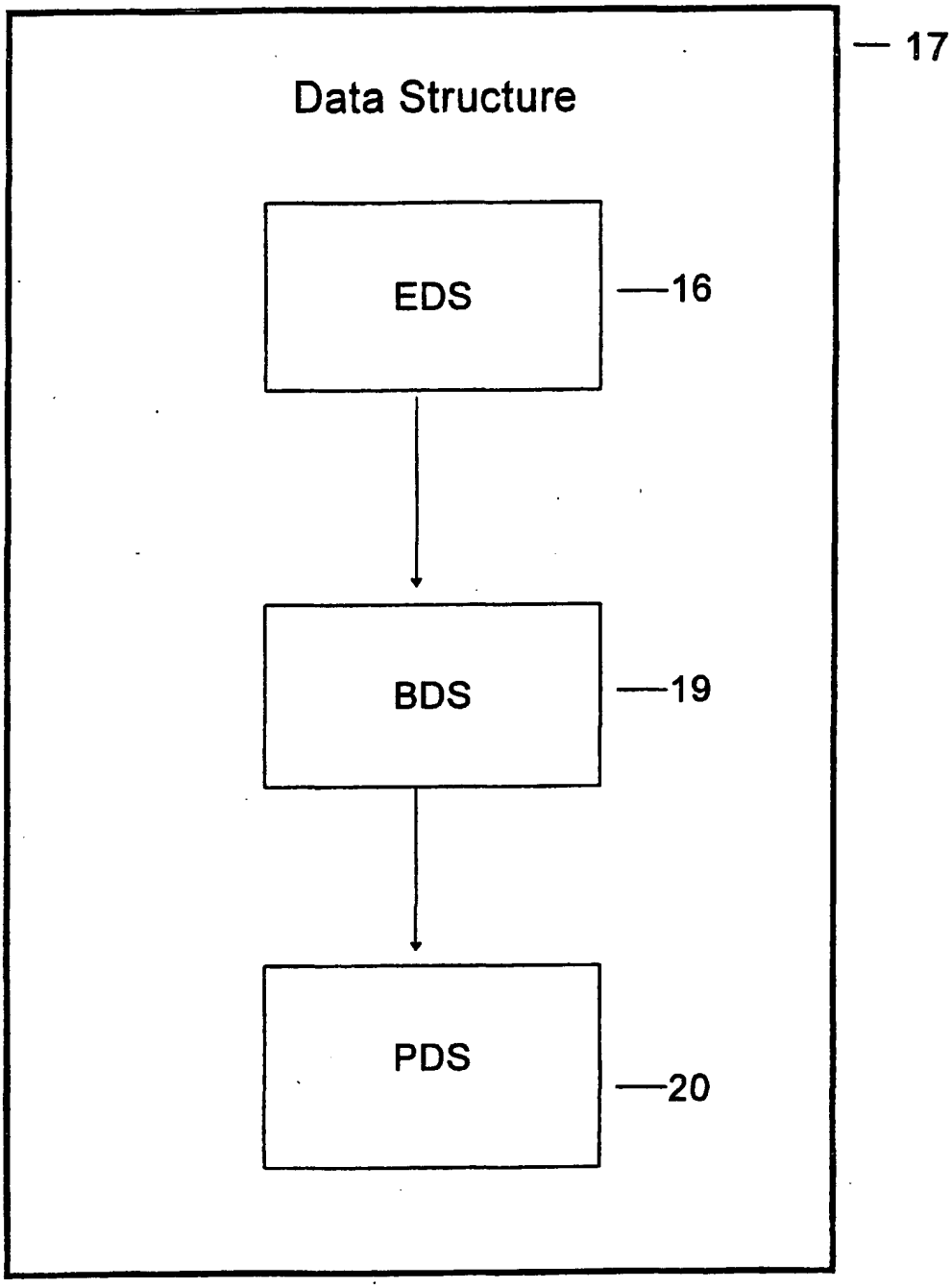


FIG. 4

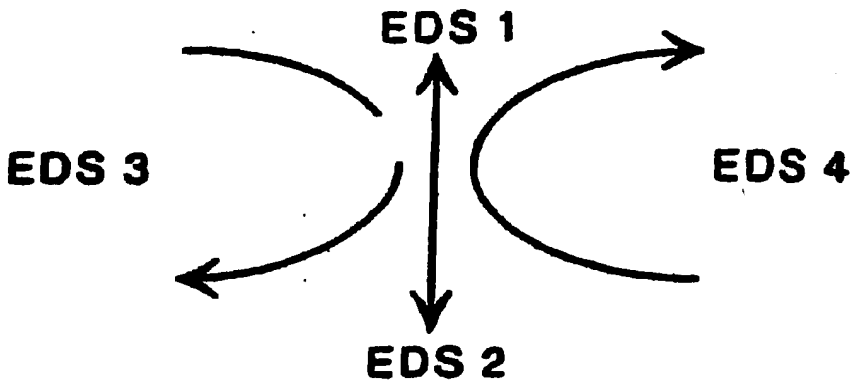


FIG. 5

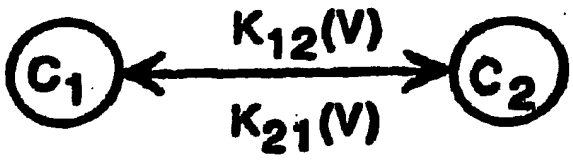


FIG. 6

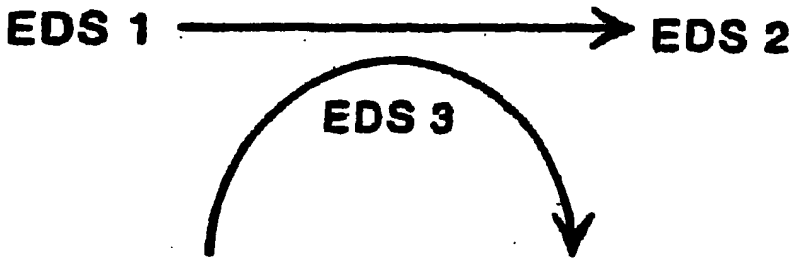


FIG. 7

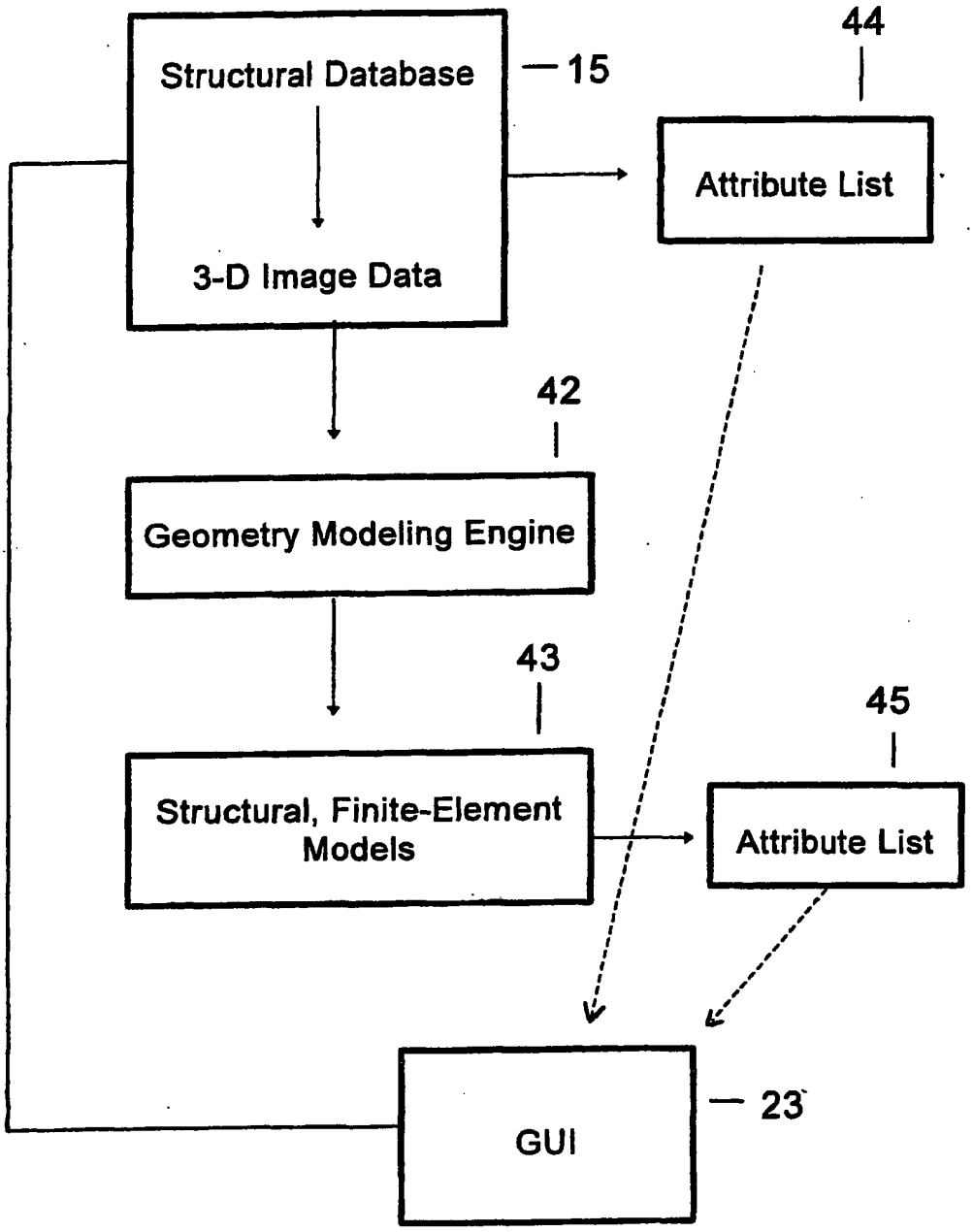

Structural Database
15
44
Attribute List
3-D Image Data
42
Geometry Modeling Engine
43
45
Structural, Finite-Element Models
Attribute List
GUI
23

FIG. 8

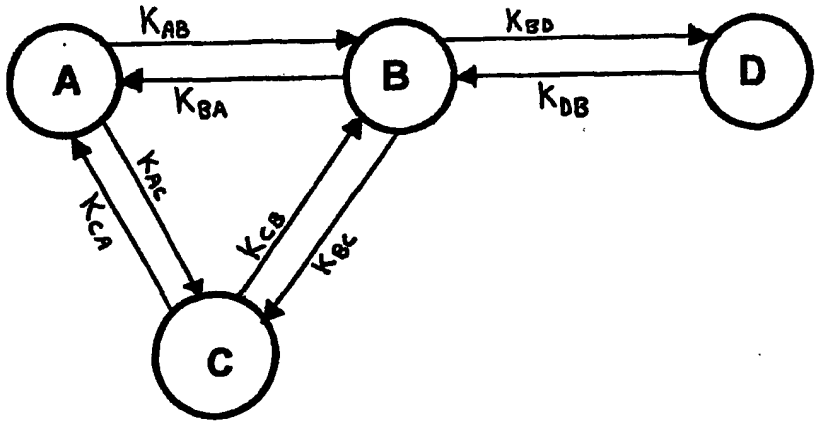


FIG. 9a

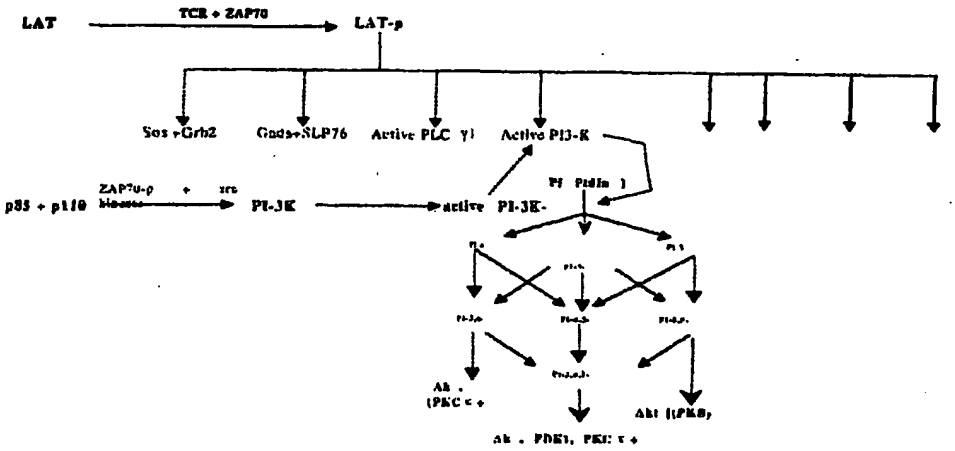

LAT
TCR + ZAP70
LAT-p
Sos +Grb2
Gads+SLP76
Active PLC γ1
Active PI3-K
p85 + p110
PI-3K
active PI-3K-
Ak .
(PKC ε +
Akt (PKB)

FIG. 9b

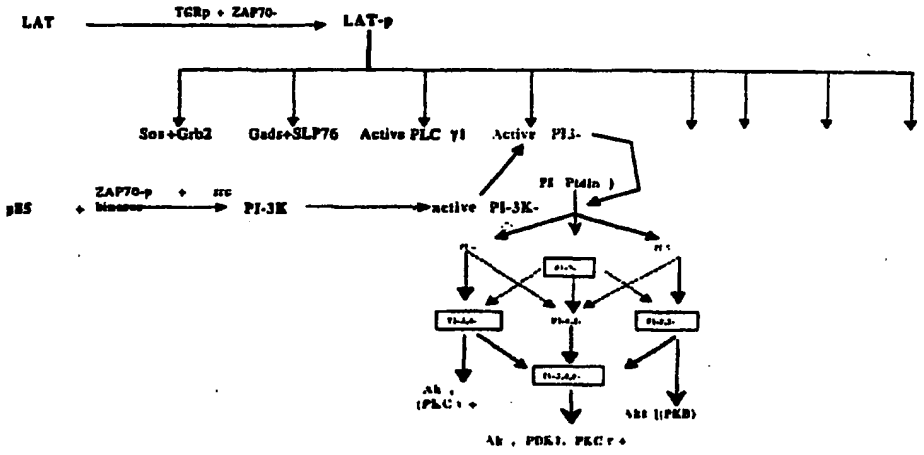

LAT
TCRp + ZAP70-
LAT-p
Sos+Grb2
Gads+SLP76
Active PLC γ1
Active PI3-
PI PtdIn)
ZAP70-p
PI-3K
active PI-3K-

FIG. 9c

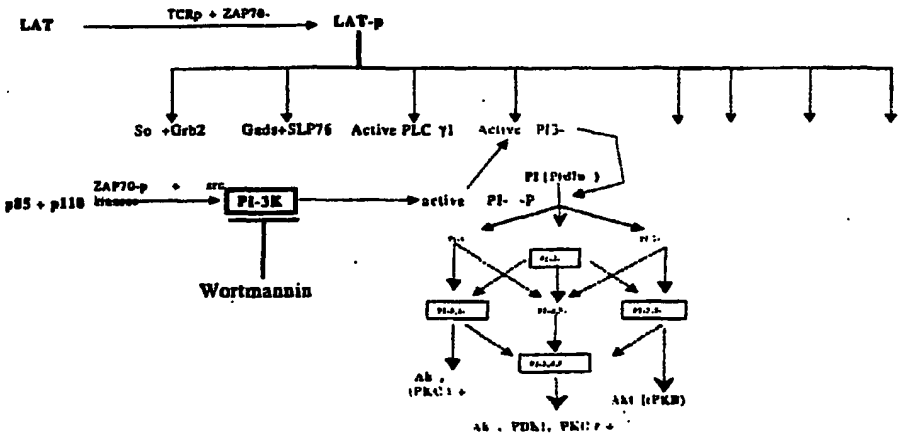

LAT
TCRp + ZAP70-
LAT-p
So +Grb2
Gads+SLP76
Active PLC γ1
Active PI3-
ZAP70-p
p85 + p110
PI-3K
active
PI- -P
Wortmannin
Akt (cPKB)

FIG. 10

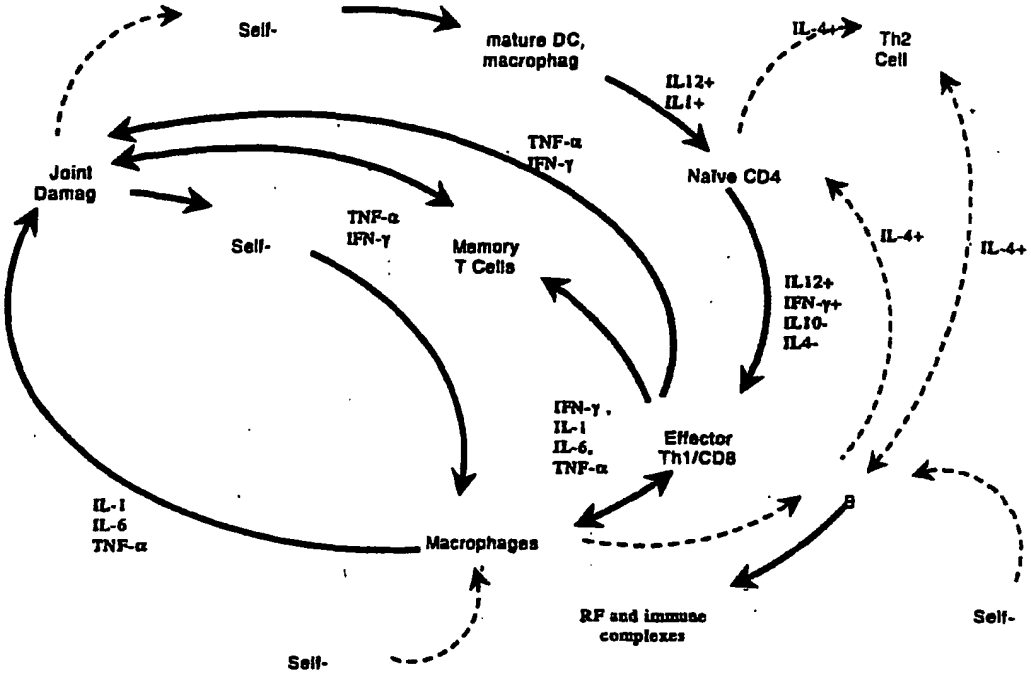

Self-
mature DC, macrophag
IL12+ IL1+
IL-4+
Th2 Cell
TNF-α IFN-γ
Joint Damag
Naive CD4
TNF-α IFN-γ
Self-
Memory T Cells
IL-4+
IL-4+
IL12+ IFN-γ+ IL10- IL4-
IFN-γ, IL-1 IL-6, TNF-α
Effector Th1/CD8
IL-1 IL-6 TNF-α
Macrophages
B
RF and immune complexes
Self-
Self-

FIG. 11

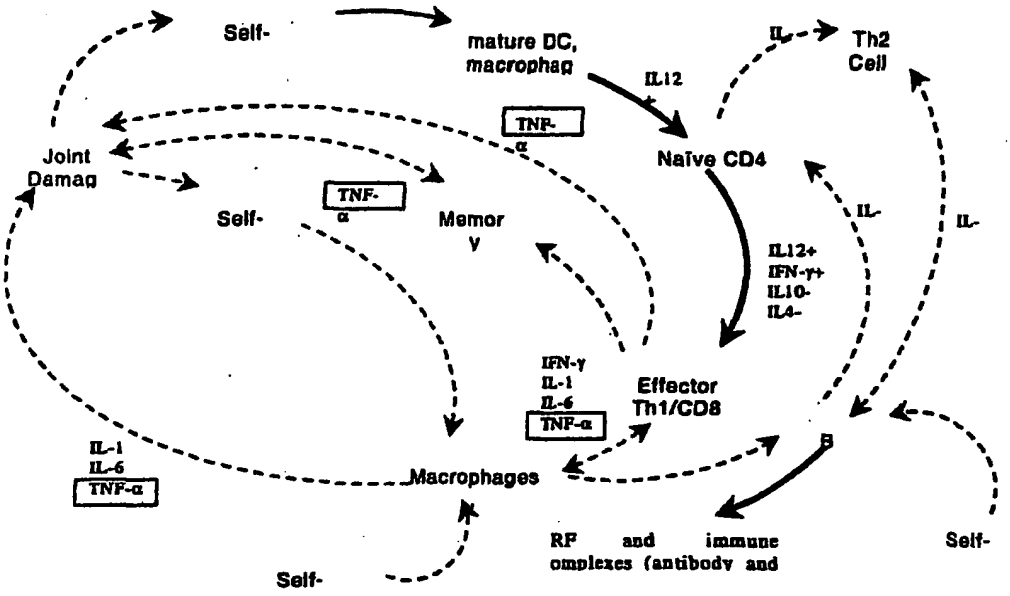

Self-
mature DC,
macrophag
IL12
+
IL-
Th2
Cell
TNF-
α
Joint
Damag
Naïve CD4
TNF-
α
Self-
Memor
y
IL-
IL-
IL12+
IFN-γ+
IL10-
IL4-
IFN-γ
IL-1
IL-6
TNF-α
Effector
Th1/CD8
IL-1
IL-6
TNF-α
Macrophages
B
RF and immune
omplexes (antibody and
Self-
Self-

FIG. 12a

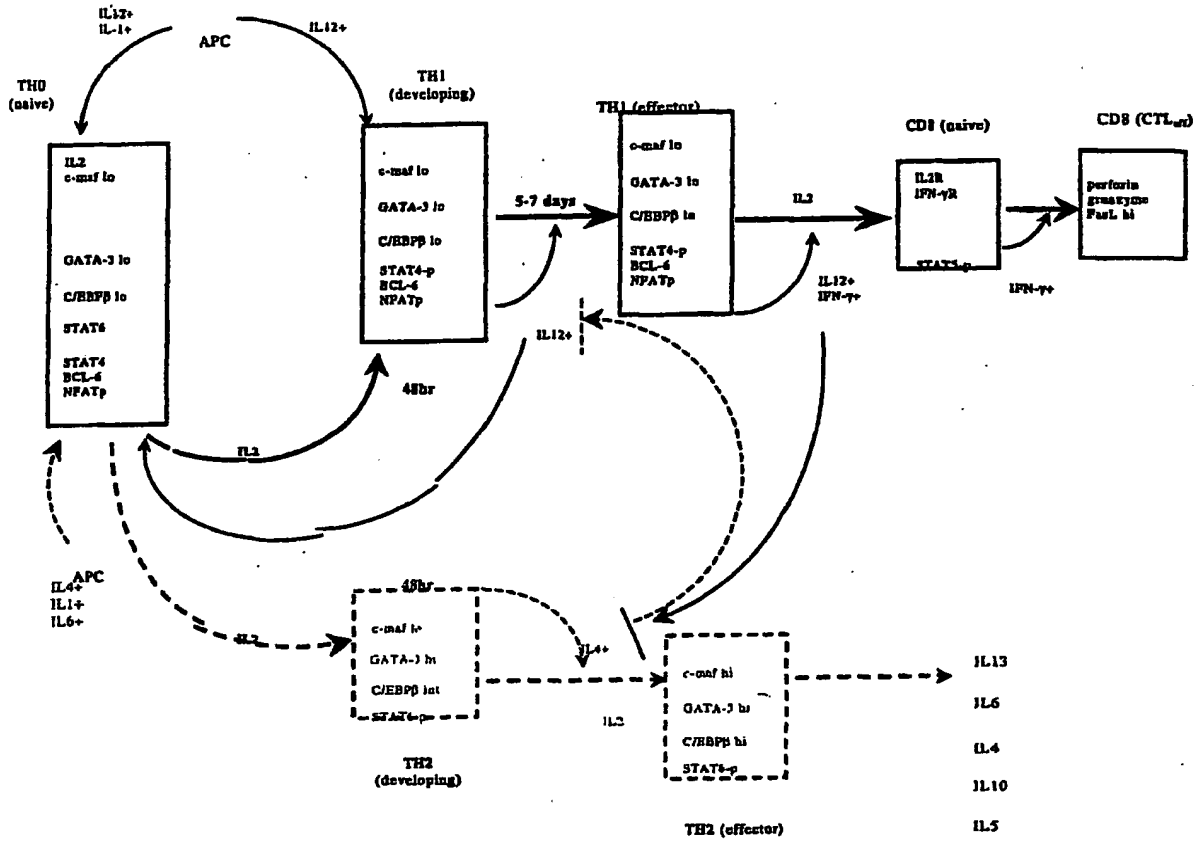

IL12+
IL-1+
APC
IL12+
TH0
(naive)
TH1
(developing)
TH1 (effector)
CD8 (naive)
CD8 (CTL)
IL2
c-maf lo
GATA-3 lo
C/EBPβ lo
STAT4
STAT4
BCL-6
NFATp
c-maf lo
GATA-3 lo
C/EBPβ lo
STAT4-p
BCL-6
NFATp
5-7 days
c-maf lo
GATA-3 lo
C/EBPβ lo
STAT4-p
BCL-6
NFATp
IL2
IL2R
IFN-γR
perforin
granzyme
FasL hi
IL12+
IFN-γ+
IFN-γ+
48hr
IL2
IL12+
APC
IL4+
IL1+
IL6+
48hr
c-maf lo
GATA-3 hi
C/EBPβ lat
STAT4-p
IL4+
IL2
c-maf hi
GATA-3 hi
C/EBPβ hi
STAT4-p
TH2
(developing)
TH2 (effector)
IL13
IL6
IL4
IL10
IL5

FIG. 12b

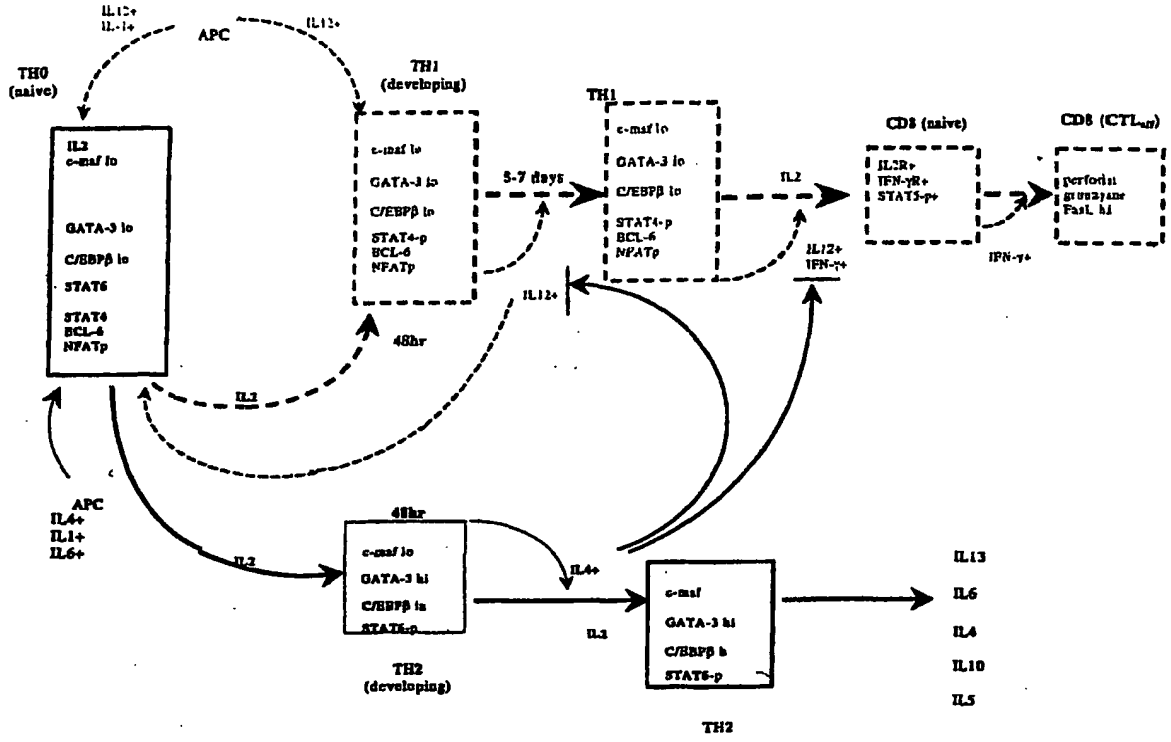

IL12+
IL-1+
APC
IL12+
TH0
(naive)
IL2
c-maf lo
GATA-3 lo
C/EBPβ lo
STAT6
STAT4
BCL-6
NFATp
TH1
(developing)
c-maf lo
GATA-3 lo
C/EBPβ lo
STAT4-p
BCL-6
NFATp
5-7 days
TH1
c-maf lo
GATA-3 lo
C/EBPβ lo
STAT4-p
BCL-6
NFATp
IL2
CD8 (naive)
IL2R+
IFN-γR+
STAT5-p+
CD8 (CTL$_{eff}$)
perforin
granzyme
FasL hi
IFN-γ+
IL12+
IFN-γ+
IL12+
48hr
IL2
APC
IL4+
IL1+
IL6+
IL2
48hr
c-maf lo
GATA-3 hi
C/EBPβ lo
STAT6-p
TH2
(developing)
IL4+
IL2
c-maf
GATA-3 hi
C/EBPβ lo
STAT6-p
TH2
IL13
IL6
IL4
IL10
IL5

FIG. 13

Question: Reported that Vav SH2 domain binds to phospho-Y315 in ZAP-70, and this binding event is required for the tyrosine phosphorylation and function of Vav. Does this mean that Vav binds phospho-ZAP independent of SLP-76/LAT association? Or that (independently) LAT/SLP-76/vav then *allows* vav phosphorylation by ZAP?

Vav: [proto-oncogene, GEF like Sos; (1 SH2, 2 SH3, 1 Dbl (DH), 1CH, 1 PH] associated with phospho-SLP-76 in T cell activation (cytoskeletal rearrangement). Generally, affects in GDP ↔ GTP exchange. **PH domain:** pleckstrin homology: facilitate membrane localization; **CH domain:** calponin homology: involved in actin binding; **DH domain:** *proto-oncogene* Dbl homology: confers capacity for nucleotide exchange for the rho family of GTPases which regulate cytoskeletal organization and JNK activity. Vav may also affect other rho GTPase members and most likely also the Rac1 pathway (and Cdc42. *Note: apparently cytoskeletal rearrangements also involve Ca elevation but do not involve ras activation.*

Role in Cytokine Therapeutics: Vav-SLP-76 and transcription: in contrast to transcription block seen after decreasing the number of LAT/GRB2(Grap) associations; decreasing SLP-76/vav or SLP/Nck association only partially inhibits transcription...suggesting synergistic (rather that obligatory interactions)...since IL-2 transcription still proceeds in the absence of SLP-76/vav.

Role in Transplantation?: vav and NK cells: affects adhesion, granule exocytosis, and cellular cytotoxicity. Vav/Rac1 activity results in cytoskeletal modifications, mediating natural killing (NK) activity after adhesion and granule exocytosis.

Gene Modulation: **Vav-/- mice** have severe defects in positive and negative selection of thymocytes, and are resistant to peptide and CD3/CD28 induced cell death, indicating that vav is upstream of mitochondrial pore opening and caspase activation in AICD. Vav deficiency also blocks TCR aggregation since actin reorganization is affected.

FIG. 14

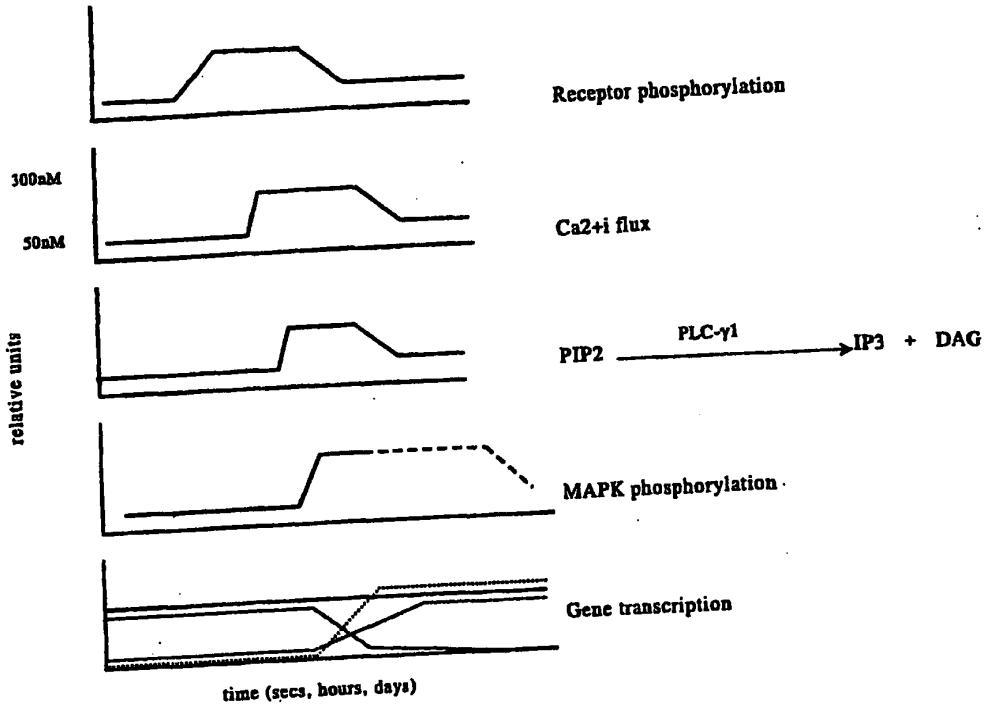
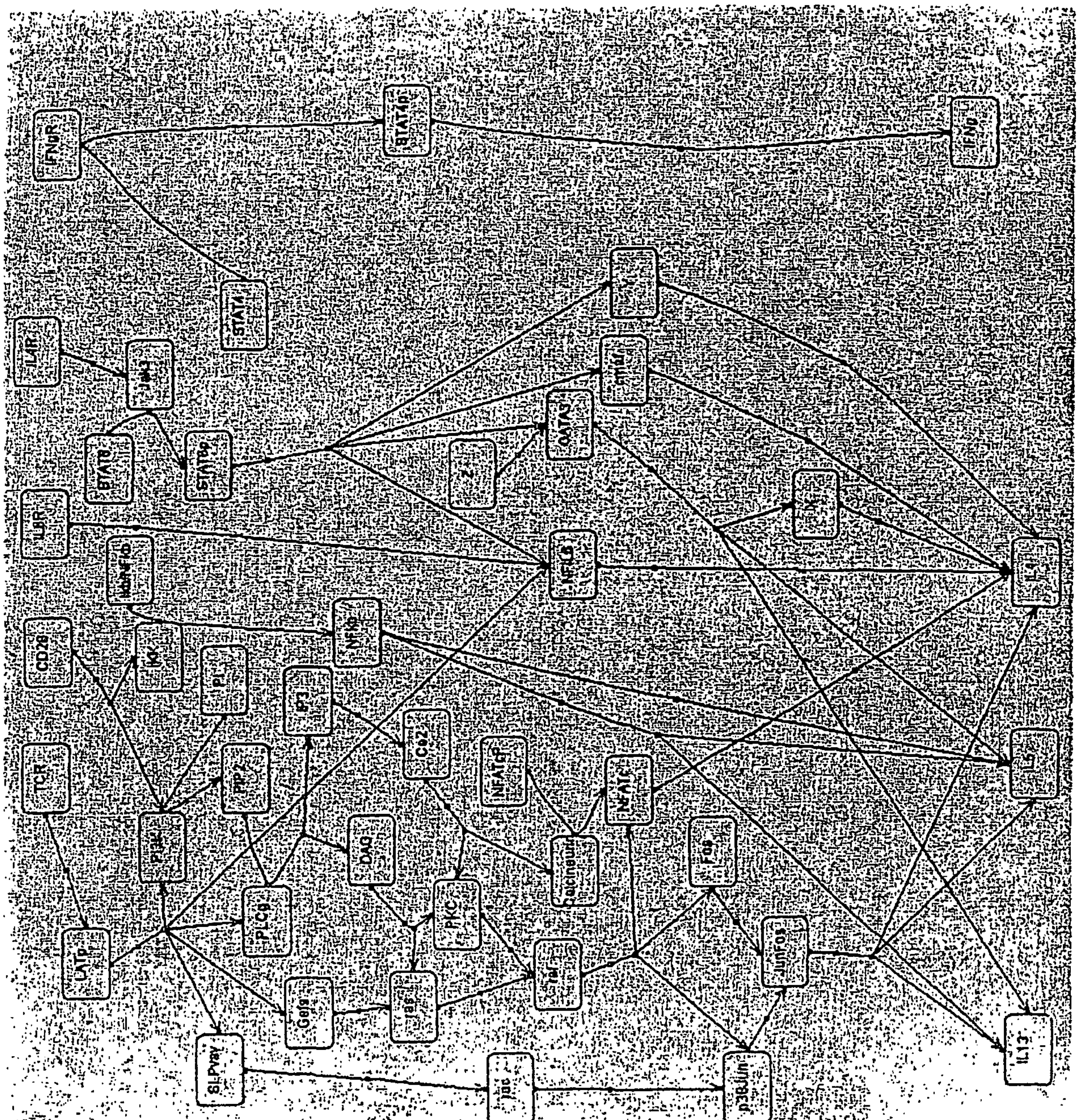

Receptor phosphorylation
300nM
50nM
Ca2+i flux
PIP2
PLC-γ1
IP3 + DAG
relative units
MAPK phosphorylation
Gene transcription
time (secs, hours, days)

FIG. 15


IFNgR
STAT4
IFNg
TCR
CD28
LAT
PLCg
DAG
PKC
NFATC
Fos
JunFos
GATA3
NFkB
IL13